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IN TWO VOLUMES

VOLUME II

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PREFACE TO THE SECOND EDITION

The purpose, plan, and scope of this treatise are given in the accompanying preface to the first edition.

This second edition, which represents a significant expansion of the first, contains twenty-six chapters, of which the following eight are new: the reactions of aliphatic hydrocarbons; synthetic polymers; catalytic hydrogenation and hydrogenolysis; organic sulfur compounds; aliphatic fluorides; the chemistry of the porphyrins; chlorophyll; and the redistribution reaction. All the chapters carried over from the first edition have been revised. In some chapters the literature has been reviewed up to September, 1942.

Corrections and suggestions will again be cordially welcomed. The editors are grateful to many friends for the examination of the manuscripts. Particular thanks are due to Messrs. R. K. Abbott, R. W. Leeper, D. S. Melstrom, G. J. O'Donnell, S. M. Spatz, J. R. Thirtle, and L. A. Woods.

H. G.

AMES, IOWA
October, 1942

PREFACE TO THE FIRST EDITION

Organic chemistry is richly endowed with excellent textbooks. However, there is a need for a general treatise of organic chemistry suitable for instruction at the graduate level. Such a book must focus attention upon new developments. At best, it can but serve the purpose of the moment and provide a point of departure for unceasing revision.

The idea of a collaborative work by specialists in the several branches of the science was developed in 1934. Each author was asked to prepare a chapter dealing with a subject of particular interest to himself. It was hoped to obtain, in this way, an authoritative treatise which would cover most of the important phases of organic chemistry. The execution of this plan has resulted in the present volumes.

For the sake of convenience in revising and expanding the book, the rapidly developing fields of natural products, relationship between physical properties and chemical constitution, valence, and resonance have been grouped together in the second volume. It is planned to revise both volumes at intervals, not only in order to bring the present material up to date, but also to permit the inclusion of new chapters to fill the more conspicuous gaps. For example, chapters on polymerization and chlorophyll will be included in the next edition. Corrections and suggestions will be heartily welcomed.

The contents have been integrated and the accessibility of the information increased by cross references, by individual tables of contents for each chapter, and by a comprehensive subject index which is repeated in each of the two volumes. The inordinate wealth of the literature has made it necessary to restrict references, in general, to a relatively few selected original articles. Researches are cited, as a rule, by reference to the most recent publications; however, sufficient references to early work are given to provide an historical background. Occasional chapters, particularly those in the field of natural products, have abundant citations to original articles, and should be especially useful to research workers. In some chapters the literature has been reviewed up to September, 1937. There is, in addition, occasional mention of work hitherto unpublished. The section General References at the end of each chapter includes mention of some of the more important review articles and books as a guide to collateral reading.

The editors gratefully acknowledge the assistance of many friends in the examination of the manuscripts. Valuable aid was provided by the late Dr. W. H. Carothers, who served on the Editorial Board. Special thanks are due to Drs. G. E. Hilbert, J. F. Nelson, P. T. Parker, A. M. Patterson, G. F. Wright, and Messrs. J. C. Bailie, R. L. Bebb, L. C. Cheney, E. J. Crane, W. Harber, A. L. Jacoby, and J. Swislowsky.

H. G.

AMES, IOWA
December, 1937

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VOLUME I

CHAPTER 14

NATURAL AMINO ACIDS

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CONTENTS

AMINO ACIDS FROM PROTEINS

The term protein connotes an ill-defined group of complex nitrogenous organic substances which form an important part of animal and vegetable tissues. The separation and characterization of individual "simple" proteins depend mainly on solubility relations, in accordance with which they are classified as albumins, globulins, and so forth. All the simple proteins yield ammonia and mixtures of amino acids on hydrolysis by acids, alkalies, or enzymes. "Conjugated" proteins also exist; these yield, besides amino acids, other products such as purines, pyrimidines, porphyrins, carbohydrates (or their derivatives), lipoidal substances, and phosphoric acid. Invariably, however, the principal products of hydrolysis consist of amino acids.

The most convenient method of hydrolysis involves treatment with

hot aqueous mineral acids. The action of hot alkalies, though it readily brings about the desired hydrolysis, is less satisfactory, for during the process a notable proportion of the amino acids, which preëxist in pure optically active form, become racemized. This objection applies in a far less degree to acid hydrolysis. The action of proteolytic enzymes, though offering the practical disadvantage of slow and often incomplete action, induces neither racemization nor decomposition of the more sensitive amino acids.

In proteins the constituent amino acids are united by peptide linkages (—CO–NH— or, with the prolines (pp. 1118, 1120), —CO—N <), which on hydrolysis are opened with liberation of carboxyl and amino or imino groups. To follow the progress of hydrolysis, three methods are available: (1) titration of carboxyl groups, (2) titration of amino groups, (3) estimation of primary amino groups by treatment with nitrous acid. In the first two, conditions are so selected that the titration end points are influenced only by the groups to be estimated; in the third, a specific reaction is involved. The principles underlying the various procedures will be discussed later. On completion of hydrolysis, the resulting amino acids may be separated into three broad classes, which depend upon the preponderatingly acidic, basic, or neutral character of their members.

The predominantly acidic group consists of the monoamino dicarboxylic acids. These may be separated from the others by taking advantage either of the sparing solubility of their calcium or barium salts in aqueous alcohol, or of their selective tendency to migrate toward the positive pole when subjected in solution at suitable pH levels to the influence of an electric current.[1]

The members of the predominantly basic group, comprising the diamino monocarboxylic acids, are characterized by their precipitability with phosphotungstic acid and by their tendency to migrate towards the negative pole in neutral solution.[2] The essentially neutral monoamino monocarboxylic acids, which constitute the major portion of most protein hydrolysates, differ from the members of the other groups [*] by the fact that they can be extracted from neutral solution by butyl alcohol.[3] The majority of the members of this group, though appreciably soluble in butyl alcohol saturated with water, are insoluble in the anhydrous alcohol. Two amino acids of protein origin (proline and hydroxyproline), however, are distinguished by their solubility in pure

---

[1] Foster and Schmidt, *J. Biol. Chem.*, **56**, 545 (1923).

[2] Foster and Schmidt, *J. Am. Chem. Soc.*, **48**, 1709 (1926).

[*] Histidine (p. 1151), in which the imidazole group possesses extremely weakly basic properties, forms an exception as it accompanies the monoamino monocarboxylic acids.

[3] Dakin, *Biochem. J.*, **12**, 290 (1918); *J. Biol. Chem.*, **44**, 499 (1920).

alcohols; these also differ from all others in being not primary, but cyclic secondary amines. The group of "natural" monoamino monocarboxylic acids also includes a few which may be separated by virtue of their low solubility in water.

The following list, arranged on the basis of the above practical classification, enumerates the amino acids which have been demonstrated to be products of the hydrolysis of proteins.[4]

I. *Monoamino Dicarboxylic Acids.*

Aspartic acid	$HO_2C-CH_2-CH(NH_2)-CO_2H$
Glutamic acid	$HO_2C-CH_2-CH_2-CH(NH_2)-CO_2H$
Hydroxyglutamic acid	$HO_2C-CH_2-CHOH-CH(NH_2)-CO_2H$

II. *Basic Amino Acids.*

Arginine	$NH_2C(=NH)-NH-CH_2-CH_2-CH_2-CH(NH_2)-CO_2H$
Lysine	$NH_2-CH_2-CH_2-CH_2-CH_2-CH(NH_2)-CO_2H$

Histidine

$$CH$$
$$N \diagup \diagdown NH$$
$$CH=C-CH_2-CH(NH_2)-CO_2H$$
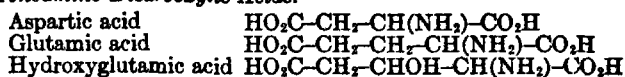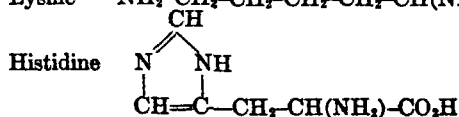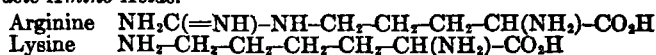
III. *Monoamino Monocarboxylic Acids.*

1. Extractable by wet butyl alcohol; readily soluble in water; insoluble in anhydrous alcohols.

Glycine	$CH_2(NH_2)-CO_2H$
Alanine	$CH_3-CH(NH_2)-CO_2H$
Valine	$(CH_3)_2CH-CH(NH_2)-CO_2H$
Leucine	$(CH_3)_2CH-CH_2-CH(NH_2)-CO_2H$
Norleucine	$CH_3-CH_2-CH_2-CH_2-CH(NH_2)-CO_2H$
Isoleucine	$CH_3-CH_2-CH(CH_3)-CH(NH_2)-CO_2H$
Phenylalanine	$C_6H_5-CH_2-CH(NH_2)-CO_2H$
Serine	$CH_2OH-CH(NH_2)-CO_2H$
Threonine	$CH_3-CHOH-CH(NH_2)-CO_2H$
Cysteine	$CH_2(SH)-CH(NH_2)-CO_2H$
Methionine	$CH_2(SCH_3)-CH_2-CH(NH_2)-CO_2H$

Tryptophan

2. Sparingly soluble in water.

Tyrosine

Diiodotyrosine

Thyroxine

Cystine $[-S-CH_2-CH(NH_2)-CO_2H]_2$

[4] Vickery and Schmidt, *Chem. Rev.*, 9, 169 (1931).

3. Soluble in alcohols.

Proline

$$CH_2\!-\!CH_2$$
$$|\qquad\quad|$$
$$CH_2\quad CH\!-\!CO_2H$$
$$\diagdown\!\diagup$$
$$NH$$

Hydroxyproline

$$CHOH\!-\!CH_2$$
$$|\qquad\quad|$$
$$CH_2\quad CH\!-\!CO_2H$$
$$\diagdown\!\diagup$$
$$NH$$
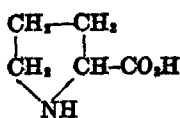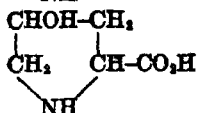
In addition to the above compounds of protein origin, certain other amino acids have been isolated from natural sources. Some of these will be discussed later.

The inclusion of cysteine in the above list is unconventional, as during the customary processes of isolation the sulfhydryl becomes oxidized to the disulfide, so that cysteine finally appears as cystine. The unquestionable presence of sulfhydryl groups in many proteins,[5] however, points to the probability of the existence of cysteine as a component amino acid.

The character, both physical and chemical, of proteins and peptides is largely determined by the nature and relative abundance of the various types of constituent amino acids. The polypeptides synthesized by Fischer contained only monoamino monocarboxylic acids, and the only free acidic and basic groups present were those terminating the peptide chain. Proteins and natural polypeptides contain polar groups, situated at the uncombined ends of the acid and basic amino acids distributed throughout the molecule; the properties of the proteins represent a resultant of the individual and mutual effects of these.

By acid hydrolysis, proteins yield considerable amounts of ammonia. There is reason to believe that this is derived from acid amide groups associated with the combined dicarboxylic acids, semi-amides of which have been isolated from the products of enzymatic hydrolysis of proteins.

Separation of the individual members of the first and second groups is effected by special methods involving selective precipitation of salts formed with metals or with acids. The quantitative aspects of these separations have been most completely developed for the basic amino acids (p. 1138). The separation of the relatively simple monoamino monocarboxylic acids is rendered particularly difficult by the familial similarity of members of homologous series, and it has not yet been found possible to develop quantitatively reliable methods for all. The original procedure of Fischer, fractional distillation of the ethyl esters under reduced pressure, involves notable losses due to formation of diketopiperazines.[6] Attempts have been made to avoid this difficulty by acylation

[5] Mirsky and Anson, J. Gen. Physiol., 18, 307 (1935).
[6] Fischer, Ber., 34, 433 (1901); Z. physiol. Chem., 33, 151 (1901); Foreman, Biochem. J., 13, 378 (1919).

of the esters before fractionation,[7] but these modifications await development to a state of practical utility.

Partial separation of the amino acids of a protein hydrolysate can be effected by taking advantage of the differential solubilities of their copper salts in water and in methyl alcohol.[8]

Advantage can also be taken[9] of the constancy of the solubility product of sparingly soluble salts of amino acids and certain strong acids in order to determine the amounts of the former in protein hydrolysates. For this purpose, complexes, such as potassium trioxalatochromiate,[10] sodium dioxpyridate,[11] or ammonium rhodanilate,[12] which precipitate specific amino acids, or simple aromatic sulfonic acids[13, 14] which yield suitable salts with a wide variety of amino acids, offer special promise.

The nutritional significance of the individual amino acids has received much study. It has long been known that rats from whose dietary protein certain amino acids were absent failed to grow normally until the missing compound was added to the ration as a supplement. Until 1935 such experimentation was hampered by the fact that normal growth could not be maintained on diets in which proteins were entirely replaced by pure amino acids. This was made possible, however, by the isolation of threonine by Rose, and his recognition of its importance as an indispensable dietary component. As a result of experiments with diets containing carbohydrates, fats, inorganic salts, glucosamine, vitamins, and various mixtures of amino acids, Rose[15] was able to establish the following classification of the amino acids according to their growth effects on immature albino rats:

INDISPENSABLE	DISPENSABLE
Arginine	Aspartic
Lysine	Glutamic
Histidine	Hydroxyglutamic
Valine	Glycine
Leucine	Alanine
Isoleucine	Norleucine
Phenylalanine	Serine
Threonine	Cystine
Methionine	Tyrosine
Tryptophan	Proline
	Hydroxyproline

[7] Cherbulies and collaborators, *Helv. Chim. Acta*, **12**, 317 (1929); **13**, 1390 (1930).
[8] Town, *Biochem. J.*, **22**, 1083 (1928); Brazier, *ibid.*, **24**, 1188 (1930).
[9] Bergmann and Stein, *J. Biol. Chem.*, **128**, 217 (1939).
[10] Bergmann and Niemann, *ibid.*, **122**, 577 (1938).
[11] Bergmann, *ibid.*, **122**, 569 (1938).
[12] Bergmann, *ibid.*, **110**, 471 (1935).
[13] Bergmann and Stein, *ibid.*, **129**, 609 (1939).
[14] Doherty, Stein, and Bergmann, *ibid.*, **135**, 487 (1940).
[15] Rose, *Physiol. Rev.*, **18**, 109 (1938).

The indispensable amino acids, listed above, fall into two further categories, namely, those of which only the stereochemically natural (p. 1085) varieties meet the needs of growth, and those of which either spatial form fulfills growth requirements:

ONLY NATURAL VARIETY PROMOTES GROWTH	EITHER CONFIGURATIONAL VARIETY PROMOTES GROWTH
Lysine	Histidine
Valine	Phenylalanine
Leucine	Methionine
Isoleucine	Tryptophan
Threonine	

Information relative to the unnatural variety of arginine is not yet available. The natural form appears to be synthesized in the body, but at too slow a rate to support growth.

It has also been found that the nutritional effect of the indispensable amino acids of the second class can be secured not only with the unnatural varieties but with certain N-acyl derivatives and α-hydroxy acids configurationally related to the natural amino acids, and with the corresponding α-keto acids. These relations, outlined for tryptophan on p. 1162, indicate that in such instances the essential portion of the molecule, i.e., that not synthesized in the animal organism, is that represented by the group R in the general formula $RCH(NH_2)CO_2H$. On the other hand, the α-amino grouping may be synthesized by normal metabolic processes from α-keto or other groupings (cf. pp. 1102, 1106) at a rate sufficient for the needs of the growing animal.

It seems probable that similar biochemical changes also proceed with the indispensable amino acids of the first class, though at rates too slow for growth requirements. Leucine of unnatural configuration is rapidly converted, in adult rats, into the natural variety. This has been demonstrated by the administration of "unnatural" leucine containing deuterium in the alkyl group and "heavy" nitrogen in the amino group; the leucine then isolated from the tissue proteins consists entirely of the natural variety and contains deuterium but practically no heavy nitrogen. However, after the administration of the corresponding isotopically labeled natural leucine, the tissue leucine contains both isotopes, the ratio of which indicates that only a minor proportion of the nitrogen had become detached in the process.[16]
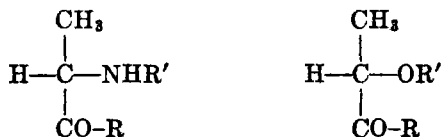
[16] Schoenheimer, Ratner, and Rittenberg. *J. Biol. Chem.*, **130**, 703 (1939); Ratner, Schoenheimer, and Rittenberg, *ibid.*, **134**, 653 (1940).

GENERAL PROPERTIES AND REACTIONS OF NATURAL AMINO ACIDS

With the exception of proline and hydroxyproline, all the amino acids isolated from protein hydrolysates contain a primary amino group in the α position to the carboxyl. The exceptions may be regarded also as α-amino acids in which the amino group is involved in ring formation; however, as may be judged from the solubility of proline in alcohol, this departure from the common form has a marked effect on physical as well as chemical properties.

With the exception of glycine, which contains no center of asymmetry, all the amino acids of protein origin occur in optically active form. The sign of rotation by which they are distinguished is conveniently that observed in hydrochloric acid solution; an amino acid which rotates to the left when dissolved in four or more equivalents of acid is stated to be the levorotatory or ($-$) variety, and vice versa.

Among the amino acids of protein origin some are dextrorotatory and some are levorotatory, but evidence is accumulating that all possess the same spatial configuration. From approximately quantitative regularities in the molecular rotatory powers of corresponding derivatives of lactic acid and alanine, Freudenberg and his collaborators have concluded [17] that natural alanine possesses the same configuration as $l(+)$-lactic acid. Analogous displacements of rotation are observed when groups (R) combined with the acid radical are varied in compounds containing the same substituents (R') on the amino and the hydroxyl group, respectively.

$$
\begin{array}{ccc}
\text{CH}_3 & & \text{CH}_3 \\
| & & | \\
\text{H--C--NHR}' & & \text{H--C--OR}' \\
| & & | \\
\text{CO-R} & & \text{CO-R}
\end{array}
$$

Similarly, the introduction of various acyl groups into natural leucine and valine and their esters causes parallel changes in optical rotation. [18]

More direct evidence for the identity of the configurations of two "natural" amino acids has been secured by Barrow and Ferguson. [19] If two optically active compounds $Cabcx$ and $Cabcy$, having the same configuration, each be converted into $Cabxy$ by replacement of a common group c by y and x, respectively, the respective products, provided that no Walden inversion has occurred, will possess opposite configurations.

[17] Freudenberg and collaborators, *Ber.*, **57**, 1547 (1924); *Ann.*, **518**, 86 (1935).
[18] Karrer and Veer, *Helv. Chim. Acta*, **15**, 746 (1932).
[19] Barrow and Ferguson, *J. Chem. Soc.*, 410 (1935).

$$
\begin{array}{ccc}
x & & x \\
| & & | \\
a\text{—}C\text{—}b & \rightarrow & a\text{—}C\text{—}b \\
| & & | \\
c & & y
\end{array}
$$

$$
\begin{array}{ccc}
y & & y \\
| & & | \\
a\text{—}C\text{—}b & \rightarrow & a\text{—}C\text{—}b \\
| & & | \\
c & & x
\end{array}
$$
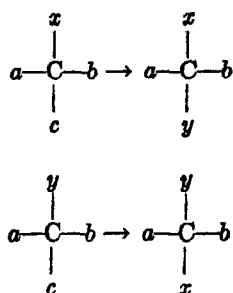
This principle has been applied to the natural, dextrorotatory forms of alanine and valine.

$$
\begin{array}{cccc}
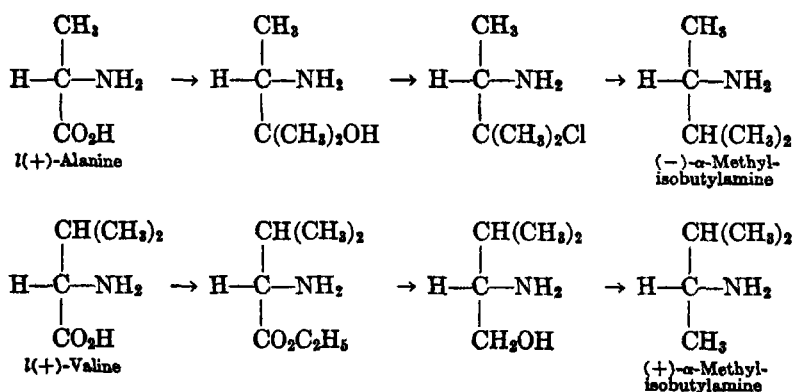CH_2 & CH_3 & CH_3 & CH_2 \\
| & | & | & | \\
H\text{—}C\text{—}NH_2 & H\text{—}C\text{—}NH_2 & H\text{—}C\text{—}NH_2 & H\text{—}C\text{—}NH_2 \\
| & | & | & | \\
CO_2H & C(CH_3)_2OH & C(CH_3)_2Cl & CH(CH_3)_2
\end{array}
$$

l(+)-Alanine   (−)-α-Methyl-isobutylamine

$$
\begin{array}{cccc}
CH(CH_3)_2 & CH(CH_3)_2 & CH(CH_3)_2 & CH(CH_3)_2 \\
| & | & | & | \\
H\text{—}C\text{—}NH_2 & H\text{—}C\text{—}NH_2 & H\text{—}C\text{—}NH_2 & H\text{—}C\text{—}NH_2 \\
| & | & | & | \\
CO_2H & CO_2C_2H_5 & CH_2OH & CH_3
\end{array}
$$

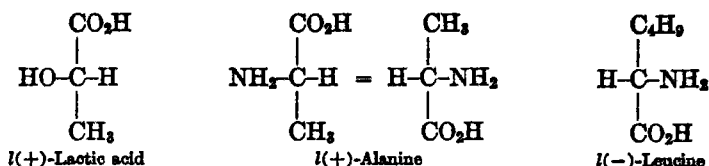l(+)-Valine   (+)-α-Methyl-isobutylamine

The α-methylisobutylamine from the natural alanine formed a levorotatory hydrochloride; that from the valine was found to be dextrorotatory. During the syntheses some loss of activity occurred in each case, but since none of the atoms directly attached to the asymmetric carbon atoms was replaced during the processes, Walden inversions (p. 264) were not to be anticipated. Natural alanine and valine therefore possess the same configuration.

The rotatory power of an amino acid is often entirely different in neutral, acid, and alkaline solution. With the natural compounds the values pass through a negative maximum at the isoelectric point and invariably become less levo- (or more dextro-) rotatory with increasing molar proportions of either alkali or acid.[20] The reverse holds for the unnatural varieties.

[20] Wood, *J. Chem. Soc.*, **105**, 1988 (1914); Clough, *ibid.*, **107**, 1509 (1915); Levene and collaborators, *J. Biol. Chem.*, **81**, 687 (1929); Lutz and Jirgensons, *Ber.*, **63**, 448 (1930); **64**, 1221 (1931).

Natural amino acids of protein origin are accordingly believed to possess universally the same configuration as $l(+)$-lactic acid, and this spatial relationship is expressed by the use of the prefix l-, which is employed without regard to the direction of the observed rotation. The latter is indicated by the sign $(+)$ or $(-)$.

$$
\begin{array}{cccc}
\text{CO}_2\text{H} & \text{CO}_2\text{H} \quad \text{CH}_3 & & \text{C}_4\text{H}_9 \\
| & | \qquad\quad | & & | \\
\text{HO-C-H} & \text{NH}_2\text{-C-H} = \text{H-C-NH}_2 & & \text{H-C-NH}_2 \\
| & | \qquad\quad | & & | \\
\text{CH}_3 & \text{CH}_3 \quad\;\; \text{CO}_2\text{H} & & \text{CO}_2\text{H} \\
l(+)\text{-Lactic acid} & l(+)\text{-Alanine} & & l(-)\text{-Leucine}
\end{array}
$$

The solubility relations of the simple α-amino monocarboxylic acids have been subjected to a critical study by Cohn and his collaborators.[21] With increasing length of chain, the solubility in water decreases and the solubility in aqueous alcohol increases. In the homologous series, the difference between the logarithms of the solubility ratios for water and for absolute alcohol decreases by a constant amount for each additional methylene group. The substantial insolubility of amino acids, in general, in absolute alcohol and other organic liquids reflects the charged condition of the molecule. In alcohol-water systems containing small proportions of alcohol the logarithm of the molar solubility diminishes inversely as the dielectric constant. The effect of inorganic salts, and the mutual effect of different amino acids, present in the same solution, upon their individual solubilities are ascribable to their influence upon the dielectric constant of the solvent.

For every amino acid there is a definite value of pH at which it fails to migrate in solution to either pole when subjected to an electric current. This value, termed the isoelectric point, is that at which the molecule as a whole carries no unbalanced positive or negative charge. The isoelectric point coincides with the point of minimum solubility.

According to the modern theory,[22] an aliphatic amino acid in solution at its isoelectric point exists in its most highly charged condition with respect to its acidic and basic groups alike. This theory alone explains, for example, the effect of formaldehyde on the titration curves of amino acids. Addition of increasing amounts of formaldehyde to a solution of glycine causes a downward displacement of the curve in the region of higher pH but no change in that of lower pH; a similar effect is observed with ammonium acetate. Since, according to generally accepted views, the effect of the addition of alkali to ammonium salts is the suppression

[21] Cohn, McMeekin, Edsall, and Weare, J. Am. Chem. Soc., **56**, 2270 (1934).
[22] Bjerrum, Z. physik. Chem., **104**, 147 (1923); Harris, Biochem. J., **24**, 1080 (1930).

of basic ionization, it follows that in glycine, as in ammonium acetate, the upper portion of the titration curve relates to the basic function. In each case, therefore, the formaldehyde similarly suppresses the dissociation of the basic groups. With amino acids containing more than one amino group (e.g., lysine) the number of constituent curves characteristically shifted by addition of formaldehyde is equal to the number of basic groups present in the amino acid molecule; conversely, with monoamino dicarboxylic acids (e.g., aspartic acid) only one segment of the original titration curve is displaced, the two attributable to the carboxyl groups remaining unaltered. On the other hand, formaldehyde brings about little or no displacement in the upper (higher pH) portion of the titration curve of p-aminobenzoic acid, from which it is concluded that the aromatic amino group is only slightly dissociated.

Aliphatic amino acids are therefore regarded as existing, in aqueous solution, largely in the form of molecules containing both positive and negative charges.

$$NH_3^+-CHR-CO_2^-$$

Such molecules, the net charge of which is zero at the isoelectric point, have received the infelicitous name "Zwitterion" (from the German word *Zwitter*, meaning hermaphrodite). The expression "dipolar ion" is more acceptable to the linguistically sensitive than the hybrid term currently employed.

A solution of any given amino acid in pure water has not necessarily the pH corresponding to the isoelectric point of the amino acid; this would be true only if the acid and basic functions had exactly the same tendency to assume the charged condition. In the simple α-amino acids, the carboxyl groups have a slightly greater tendency to part with their protons than the amino groups to accept them; as a result the hydrogen-ion concentration of their solutions is higher than that of water, but not sufficiently high to bring the total number of positive and negative charges on all the amino acid molecules into exact balance. This condition can be reached only by the addition to the solution of more hydrogen ions in the form of some acid. For the monoamino monocarboxylic acids, the isoelectric points of which lie at approximately $pH = 6$, the discrepancy between the pH value of pure aqueous solutions and isoelectric point is but slight; it is much greater, of course, with the monoamino dicarboxylic acids. Conversely, the isoelectric point of the diamino monocarboxylic acids lies above $pH = 7$, and hydroxyl ions (in the form of alkali) must be added to their pure solutions to render them isoelectric.

Addition of increasing amounts of mineral acid to a solution of an amino acid causes the suppression of the negative charge, until finally

the equilibrium mixture contains the amino acid in its purely cationic form.

$$NH_3^+-CHR-CO_2^- + H^+ \rightleftarrows NH_3^+-CHR-CO_2H$$
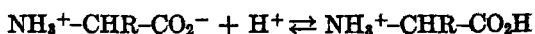
Addition of alkali causes the suppression of the positive charge, with production of the anionic form.

$$NH_3^+-CHR-CO_2^- + OH^- \rightleftarrows NH_2-CHR-CO_2^- + H_2O$$
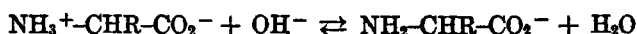
The equilibria involved at different pH levels are illustrated by the case, discussed by Cohn in his admirable review,[23] of the monoamino dicarboxylic acids.

$$pH=2 \qquad\qquad pH=4 \qquad\qquad pH=9.6$$
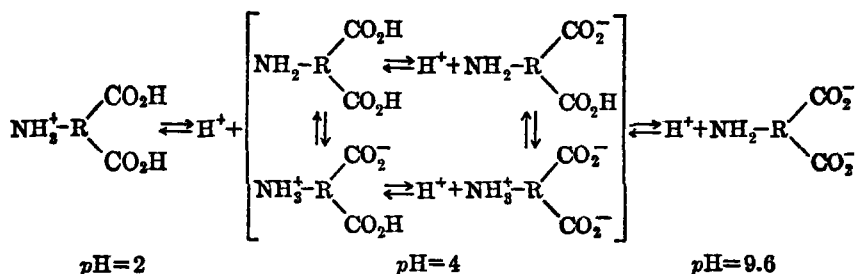
That amino acids in their isoelectric range exist mainly in the dipolar ionic form is indicated by their Raman spectra.[24] Fatty acids in aqueous solution (in which they are but weakly ionized) exhibit a line at about 1720 cm.$^{-1}$ characteristic of the carbonyl group; on the addition of sufficient alkali to cause almost complete ionization, this line vanishes. Amino acids fail to exhibit a line at this frequency, but do so when converted into their hydrochlorides. Conversely, free primary amines show strong Raman lines between 3300 and 3400 cm.$^{-1}$; lines in this region are not displayed by amino acids in their isoelectric zone, but appear on the addition of alkali. Similar conclusions may be drawn from the behavior of amino acids towards water containing isotopic oxygen (H_2O^{18}). In this medium, simple carboxylic acids acquire "heavy" oxygen atoms at pH 1, but do not so exchange when in the form of their potassium salts; glycine fails to exchange at pH 7, but does so at pH 1.9.[25] The production of betaines from ethereal diazomethane with solid amino acids also points to their existence in dipolar ionic form;[26] in most cases the simultaneous production of amino acid methyl ester demonstrates the presence

[23] Cohn, *Ergeb. Physiol.*, **33**, 781 (1931).
[24] Edsall, *J. Chem. Phys.*, **4**, 1 (1936); **5**, 225 (1937).
[25] Mears, *ibid.*, **6**, 295 (1938).
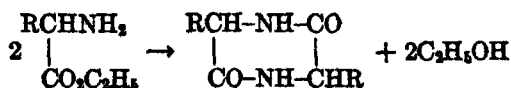[26] Kuhn and Brydówna, *Ber.*, **70**, 1333 (1937).

of some of the uncharged form. Only the ester is formed from compounds like anthranilic acid in which the amino group is inherently of weakly basic character.

Dipolar ions possess a large electric moment,[27] particularly those of lysine and arginine, which exist in solution largely in the form of ions containing positive and negative charges at opposite ends of relatively long chains. Dicarboxylic amino acids, in isoelectric solution, exist mainly as less polar ions, resembling those of the simple α-amino acids, for their terminal carboxyl groups are less highly dissociated than those contiguous to the amino group. In solvents of low dielectric constant, such as 90 per cent alcohol, the concentration of highly polar ions is smaller, and that of uncharged molecules greater, than in water. For this reason it is possible, by the use of suitable indicators, to titrate independently either the acidic [28] or the basic [29] function of amino acids in aqueous alcohol, acetone, or dioxan [30] solution.

The basic groups of amino acids can be quantitatively titrated in glacial acetic acid solution with perchloric acid in the same solvent.[31] The titration may be carried out either potentiometrically by the method of Hall and Conant,[32] or with the aid of a suitable indicator such as crystal violet.[33] The amino group behaves as a strong base, as in all aliphatic amines,[34] while the dissociation of the carboxyl group is completely suppressed by the solvent.

The dipolar character of the amino acids is reflected in their relative infusibility and low volatility. When strongly heated, they melt with profound decomposition [35] at temperatures well above 200°; some show a tendency to sublime below the decomposition point.[36]

Since the negative character of the carboxyl group is suppressed by esterification, the amino acid esters are far more volatile than the amino acids. On distillation, they undergo some condensation with loss of alcohol. This reaction occurs more readily with the methyl and ethyl esters

$$
2 \begin{array}{c} RCHNH_2 \\ | \\ CO_2C_2H_5 \end{array} \rightarrow \begin{array}{c} RCH-NH-CO \\ | \qquad\qquad | \\ CO-NH-CHR \end{array} + 2C_2H_5OH
$$

[27] Edsall and Blanchard, *J. Am. Chem. Soc.*, **55**, 2337 (1933).

[28] Foreman, *Biochem. J.*, **14**, 451 (1920); **22**, 208, 222 (1928).

[29] Linderstrøm-Lang, *Z. physiol. Chem.*, **173**, 32; **174**, 275 (1928).

[30] Popovici and Radulescu, *Bull. soc. chim. biol.*, **20**, 73 (1938).

[31] Harris, *Biochem. J.*, **29**, 2820 (1935); *J. Biol. Chem.*, **84**, 296 (1929); Nadeau and Branchen, *J. Am. Chem. Soc.*, **57**, 1363 (1935).

[32] Hall and Conant, *ibid.*, **49**, 3047 (1927).

[33] Conant and collaborators, *ibid.*, **49**, 3062 (1927); **52**, 4436 (1930).

[34] Hall and collaborators, *ibid.*, **50**, 2367 (1928); **52**, 5115 (1930).

[35] Dunn and Brophy, *J. Biol. Chem.*, **99**, 221 (1932).

[36] Brown, *Trans. Roy. Soc. Can.*, Sect. III, **26**, 173 (1932) [*C.A.*, **27**, 1617 (1933)].

than with esters of higher alcohols such as butyl.[37] Analogous condensations take place with loss of water, when amino acids are heated alone, or better, in the presence of β-naphthol.[38]
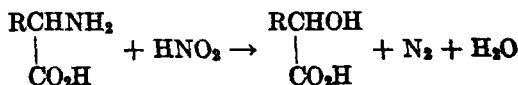
Another type of decomposition which occurs on heating is decarboxylation.[39]

$$\begin{array}{c} \text{RCHNH}_2 \\ | \\ \text{CO}_2\text{H} \end{array} \rightarrow \text{RCH}_2\text{NH}_2 + \text{CO}_2$$

This reaction takes place more readily in the presence of barium hydroxide or diphenylamine.[40] It also occurs when solutions of amino acids are exposed to the action of putrefactive organisms. Several of the amines so produced from natural amino acids are pharmacologically active; their formation in the lower intestine may be responsible for some forms of auto-intoxication.

The usual functions of the carboxyl group in an amino acid are evident only under conditions in which the negative charge is suppressed. On treatment with alcohols, esterification takes place only in the presence of an equivalent amount of a mineral acid, such as hydrogen chloride.[41] Amides are formed with great difficulty by heating amino acids with alcoholic ammonia;[42] they are somewhat more readily produced by the action of alcoholic[43] or anhydrous[44] ammonia upon amino acid esters. Chlorides of amino acids are capable of existing only in the form of salts, such as the hydrochloride. These have been prepared by treating a suspension of the amino acid in acetyl chloride with phosphorus pentachloride.[45] Esters of amino acids are reduced to the corresponding aldehydes by sodium amalgam.[46]

α-Amino acids can, under suitable conditions, be made to undergo all the chemical reactions common to aliphatic primary amines. By the action of nitrous acid, for instance, they are converted into the corresponding hydroxy acids, with liberation of nitrogen.

$$\begin{array}{c} \text{RCHNH}_2 \\ | \\ \text{CO}_2\text{H} \end{array} + \text{HNO}_2 \rightarrow \begin{array}{c} \text{RCHOH} \\ | \\ \text{CO}_2\text{H} \end{array} + \text{N}_2 + \text{H}_2\text{O}$$

[37] Morgan, J. Chem. Soc., 79 (1926).

[38] Lichtenstein, J. Am. Chem. Soc., 60, 560 (1938).

[39] Cahours, Ann., 109, 10 (1859); Schulze and Barbieri, Ber., 14, 1785 (1881); 16, 1711 (1883); Erlenmeyer and Lipp, Ann., 219, 161 (1883).

[40] Johnson and Daschavsky, J. Biol. Chem., 62, 725 (1925); Abderhalden and Gebelein, Z. physiol. Chem., 152, 125 (1926).

[41] Curtius and Goebel, J. prakt. Chem., [2] 37, 150 (1888).

[42] Heintz, Ann., 150, 67 (1869).

[43] Franchimont and Friedmann, Rec. trav. chim., 25, 75 (1906).
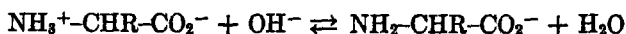
[44] Koenigs and Mylo, Ber., 41, 4427 (1908).

[45] Fischer, Ber., 38, 605, 2914 (1905).

[46] Neuberg, Ber., 41, 956 (1908); Fischer, Ber., 41, 1019 (1908).

This reaction forms the basis of a valuable analytical method [47] for the estimation of primary amino groups in amino acids, peptides, or proteins. It takes place quantitatively and rapidly by the action of acetic acid and sodium nitrite in excess; the nitric oxide which is simultaneously evolved by the reagents is removed by means of alkaline permanganate. Acid amide groups as a rule yield no nitrogen unless mineral acids are present, and ammonium salts buffered with sodium acetate react only very slowly. This reaction serves also to differentiate primary amines from the secondary variety (as in proline), from which no nitrogen is evolved. The guanidino group, which occurs in arginine, likewise yields no nitrogen unless mineral acid is present.

Comparable analytical results can be secured by treating alkaline solutions of amino acids and peptides with copper phosphate; the amount of copper taken into solution (as a complex of the ammine type) furnishes a measure of the amount of amino nitrogen present.[48] Proline and hydroxyproline, which yield no nitrogen on treatment with nitrous acid, respond to this procedure.

Acylation and similar processes are the most efficiently performed when the amino acid is in solution or in the form of a metallic salt. It seems probable that such reactions take place with amino groups only in their uncharged form, for it has been shown that in the solid state amino acids exist almost entirely in the form of electrically charged dipoles.[49] In simple aqueous solution the proportion of uncharged amino groups in equilibrium with the positively charged ionic groups is sufficient to permit acylation reactions to proceed at a slow rate; in the presence of added alkali this proportion is greatly increased, and acylation is facilitated. The principle is illustrated by the need for alkaline conditions during the introduction of phenylureido,[50] arylsulfonyl, or benzoyl groups into amino acids.[51]

$$NH_3^+-CHR-CO_2^- + OH^- \rightleftarrows NH_2-CHR-CO_2^- + H_2O$$

$$NH_2-CHR-CO_2^- + ArNCO \rightarrow ArNH-CO-NH-CHR-CO_2^-$$

In these procedures, only one such group is introduced; however, on treatment with methanesulfonyl chloride and alkali, amino acids are converted into mono- and di-N-methanesulfonyl derivatives, which are soluble in organic liquids and relatively stable towards aqueous acids and alkalies.[52]

[47] Van Slyke, *J. Biol. Chem.*, **12,** 275 (1912).

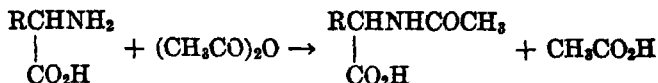[48] Pope and Stevens, *Biochem. J.*, **33**, 1070 (1939).

[49] Cohn, *Ann. Rev. Biochem.*, **4**, 93 (1935).

[50] Paal, *Ber.*, **27**, 974 (1894); Neuberg and Manasse, *Ber.*, **38**, 2359 (1905).
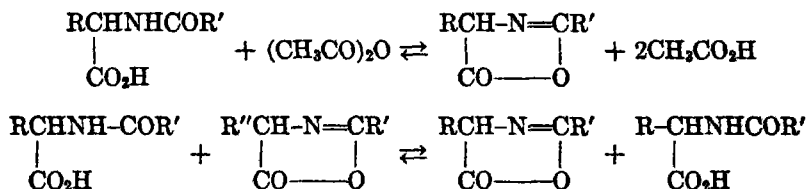
[51] Baum, *Z. physiol. Chem.*, **9**, 465 (1885); Fischer, *Ber.*, **32**, 2451 (1899); Fischer and Bergell, *Ber.*, **35**, 3779 (1902).

[52] Helferich and Grünert, *Ann.*, **545**, 178 (1940).

Acetylation of natural amino acids leads, under certain conditions, to loss of optical activity. When the reaction is carried out in cold acetic acid or in alkaline solutions by means of the theoretically necessary amount of acetic anhydride, optically active acetamino acids are produced.
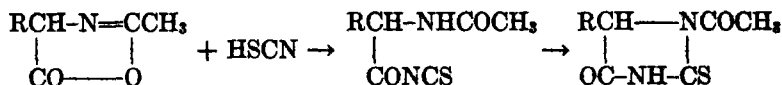
$$\begin{matrix} \text{RCHNH}_2 \\ | \\ \text{CO}_2\text{H} \end{matrix} + (\text{CH}_3\text{CO})_2\text{O} \rightarrow \begin{matrix} \text{RCHNHCOCH}_3 \\ | \\ \text{CO}_2\text{H} \end{matrix} + \text{CH}_3\text{CO}_2\text{H}$$

These are, however, racemized by heating in acetic acid solution with small quantities of acetic anhydride, and with large amounts they are converted into inactive azlactones.[53] Not only is this form of racemization brought about by acetic anhydride, but it takes place even more rapidly by the agency of other azlactones.[54] Equilibria of the types probably occur, in which the racemization takes place with the intermediary azlactones.

$$\begin{matrix} \text{RCHNHCOR}' \\ | \\ \text{CO}_2\text{H} \end{matrix} + (\text{CH}_3\text{CO})_2\text{O} \rightleftarrows \begin{matrix} \text{RCH–N}\text{=}\text{CR}' \\ | \qquad | \\ \text{CO}\text{-----}\text{O} \end{matrix} + 2\text{CH}_3\text{CO}_2\text{H}$$

$$\begin{matrix} \text{RCHNH–COR}' \\ | \\ \text{CO}_2\text{H} \end{matrix} + \begin{matrix} \text{R}''\text{CH–N}\text{=}\text{CR}' \\ | \qquad | \\ \text{CO}\text{-----}\text{O} \end{matrix} \rightleftarrows \begin{matrix} \text{RCH–N}\text{=}\text{CR}' \\ | \qquad | \\ \text{CO}\text{-----}\text{O} \end{matrix} + \begin{matrix} \text{R–CHNHCOR}' \\ | \\ \text{CO}_2\text{H} \end{matrix}$$

ably occur, in which the racemization takes place with the intermediary azlactones.

On the other hand, when an optically active amino acid is treated with excess of acetic anhydride in the presence of ammonium thiocyanate, an optically active acetyl thiohydantoin results.[55] The formation of this type of compound is presumably due to a reaction more rapid than the racemization of the azlactone,[56] and is analogous to the reaction between azlactones and hydrogen chloride.[57]

$$\begin{matrix} \text{RCH–N}\text{=}\text{CCH}_3 \\ | \qquad | \\ \text{CO}\text{-----}\text{O} \end{matrix} + \text{HSCN} \rightarrow \begin{matrix} \text{RCH–NHCOCH}_3 \\ | \\ \text{CONCS} \end{matrix} \rightarrow \begin{matrix} \text{RCH}\text{-----}\text{NCOCH}_3 \\ | \qquad | \\ \text{OC–NH–CS} \end{matrix}$$

tween azlactones and hydrogen chloride.[57]

$$\begin{matrix} \text{C}_6\text{H}_5\text{C}\text{=}\text{N-CHR} \\ | \qquad | \\ \text{O}\text{-----}\text{CO} \end{matrix} + \text{HCl} \rightarrow \begin{matrix} \text{C}_6\text{H}_5\text{CO–NHCHR} \\ | \\ \text{COCl} \end{matrix}$$
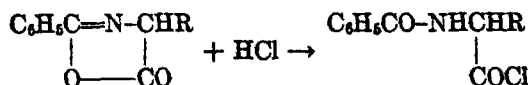
Although the acetyl group can be introduced without racemization by the action of acetic anhydride in excess upon primary amino acids dis-

[53] Bergmann and Zervas, Biochem. Z., 203, 280 (1928).
[54] Carter and Stevens, J. Biol. Chem., 133, 117 (1940).
[55] Csonka and Nicolet, ibid., 99, 213 (1932).
[56] Johnson and Scott, J. Am. Chem. Soc., 35, 1136 (1913).
[57] Mohr, Ber., 42, 2521 (1909); J. prakt. Chem., [2] 81, 49, 473; [2] 82, 322 (1910).

solved in boiling water,[58] the same reaction carried out in the presence of sodium acetate at 35–40° leads to a completely racemized acetamino acid.[59] On the other hand, under these conditions the secondary amino acid proline is acetylated without racemization.

Hydantoins of natural amino acids are rapidly racemized in cold solution by traces of alkali.[60] The racemization, which does not occur with the corresponding hydantoic acids or with hydantoins of the type is
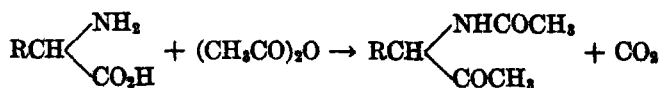
$$
\begin{array}{c}
R' \\
| \\
R-C-NH \\
| \qquad\qquad \diagdown CO \\
CO-NH \diagup
\end{array}
$$
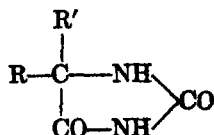
ascribed to the ionization of the hydrogen atom attached to the asymmetric carbon atom, to which activating groups are linked.[61] Introduction of a phenyl group at position 3 of the hydantoin ring

$$
\begin{array}{c}
(5)\quad\ (1) \\
RCH-NH \\
| \qquad\qquad \diagdown CO\,(2) \\
CO-NH \diagup \\
(4)\quad\ (3)
\end{array}
$$
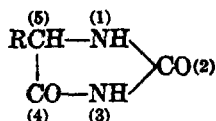
increases the rate of racemization by small quantities of alkali, and a similar, though smaller, effect occurs when a methyl group is introduced at position 1. As these substitutions prevent enolization at these positions, and thereby inhibit any conjugation with enolic unsaturation between positions 4 and 5, it is concluded that racemization cannot be due exclusively to enolization.

When primary α-amino acids are warmed with acetic anhydride in the presence of pyridine,[62] methyl acetaminoalkyl ketones are produced.

$$
RCH \diagdown \begin{array}{c} NH_2 \\ CO_2H \end{array} + (CH_3CO)_2O \rightarrow RCH \diagdown \begin{array}{c} NHCOCH_3 \\ COCH_3 \end{array} + CO_2
$$

Under the same conditions proline and N-alkylamino acids are merely acetylated, and no corresponding ketone is formed from α-amino-α-phenylpropionic acid.

[58] Behr and Clarke, *J. Am. Chem. Soc.*, **54**, 1630 (1932).
[59] du Vigneaud and Meyer, *J. Biol. Chem.*, **98**, 295; **99**, 143 (1932).
[60] Dakin, *Am. Chem. J.*, **44**, 48 (1910).
[61] Bovarnick and Clarke, *J. Am. Chem. Soc.*, **60**, 2426 (1938).
[62] Levene and Steiger, *J. Biol. Chem.*, **74**, 689 (1927); **79**, 95 (1928); Dakin and West, *ibid.*, **78**, 91, 757 (1928).
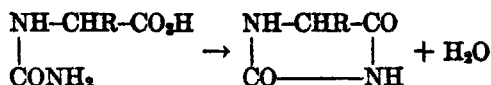
On treatment with a boiling solution of urea, α-amino acids yield the corresponding hydantoic acids.[63]

$$NH_2CONH_2 + NH_2CHR\text{-}CO_2H \rightarrow NH_2CONH\text{-}CHR\text{-}CO_2H + NH_3]$$
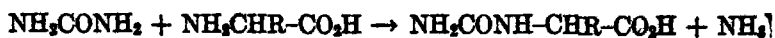
During the process racemization takes place. This may be avoided by the use of potassium cyanate.[60]

$$KCNO + NH_2CHR\text{-}CO_2H \rightarrow NH_2CONH\text{-}CHR\text{-}CO_2K$$
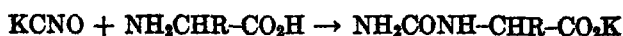
The resulting hydantoic acids readily undergo ring closure to the hydantoins on boiling with hydrochloric acid,

$$
\begin{array}{ccc}
\text{NH-CHR-CO}_2\text{H} & & \text{NH-CHR-CO} \\
| & \rightarrow & | \qquad\qquad | \quad + \text{H}_2\text{O} \\
\text{CONH}_2 & & \text{CO}\text{---------NH}
\end{array}
$$

a reaction which would proceed in the reverse direction if the reacting groups were not held near to each other by a connecting chain.

The introduction of acyl or ureido groups abolishes the amphoteric character of amino acids; the products are soluble in non-polar organic liquids, and are suitable derivatives for identification.

When carbon dioxide is passed into alkaline solutions of amino acids, salts of corresponding carbamino acids are produced.[64]

$$
\begin{array}{ccc}
\text{RCH-CO}_2^- & & \text{RCH-CO}_2^- \\
| \qquad + \text{CO}_2 + \text{OH}^- \rightarrow & & | \qquad + \text{H}_2\text{O} \\
\text{NH}_2 & & \text{NH-CO}_2^-
\end{array}
$$
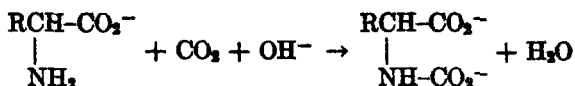
The calcium and barium salts are sparingly soluble in dilute alcohol; in boiling water they break down into the amino acids and metal carbonate. An attempt has been made [65] to exploit the differences in solubility of the barium salts of the carbamino acids for the systematic separation of the products of protein hydrolysis.

Basic mercury salts of carbamino acids are formed when mercuric acetate is added to solutions of amino acids made—and maintained—alkaline with sodium carbonate.[66]

$$
\begin{array}{ccccc}
\text{RCH-CO}_2\text{H} & & \text{R-CH-CO}_2\text{Na} & & \text{RCH-CO}_2 \\
| \qquad + \text{Na}_2\text{CO}_3 \rightarrow & & | & \rightarrow & | \qquad\qquad \rangle\text{Hg}\cdot\text{HgO} \\
\text{NH}_2 & & \text{NHCO}_2\text{Na} & & \text{NH-CO}_2
\end{array}
$$
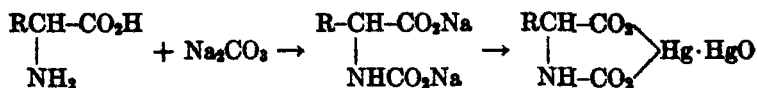
In most instances these salts are nearly quantitatively precipitated on the addition of alcohol.

[63] Lippich, Ber., 39, 2953 (1906); 41, 2974 (1908).

[64] Siegfried and collaborators, Z. physiol. Chem., 44, 85 (1905); 46, 401 (1905); 54, 423 (1908); 65, 295 (1910); 81, 260 (1912); Ber., 39, 397 (1906); Stadie and O'Brien, J. Biol. Chem., 112, 723 (1936).

[65] Schryver and collaborators, Biochem. J., 15, 636 (1921); 18, 1070 (1924).

[66] Neuberg and Kerb, Biochem. Z., 40, 498 (1912).

Amino acids are converted into the corresponding guanidino acids by treatment with O-methylisourea,[67] or S-methylisothiourea.

$$\underset{\underset{NH_2}{|}}{\overset{\overset{NH}{||}}{CH_3OC}} + NH_2-CHR-CO_2^- \rightarrow CH_3OH + \underset{\underset{NH_2}{|}}{\overset{\overset{NH}{||}}{C}}-NH-CHR-CO_2^-$$
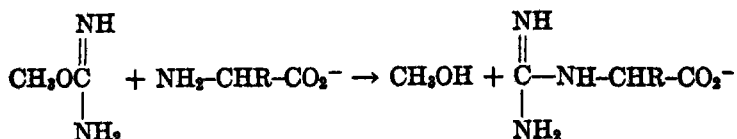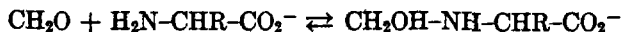
Reference has already been made (p. 1087) to the use of formaldehyde in the titration of amino acids. Sörensen,[68] who developed the process as an analytical method, ascribed the suppression of the basic functions of the amino group to the establishment of an equilibrium involving methylene compounds of the type formulated by Schiff.

$$CH_2O + NH_2-CHR-CO_2^- \rightarrow CH_2=N-CHR-CO_2^- + H_2O$$
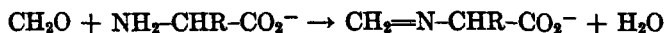
However, the reaction appears to be more complicated. Metallic salts of such condensation products have been prepared which contain the elements of one or more additional molecules of water or formaldehyde,[69] so that it seems probable that in solution an equilibrium exists between the methylene, methylol, dimethylol, and more complex forms. On the other hand, determination of the equilibrium constant of the reaction between amino acids and formaldehyde[70] points to the formation, over the range pH 8 to 10, of equimolar compounds only.

$$CH_2O + NH_2-CHR-CO_2^- + OH^- \rightleftarrows {}^-OCH_2-NH-CHR-CO_2^- + H_2O$$
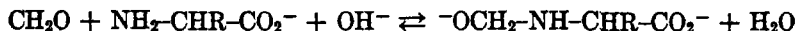
Studies of titration curves[71] of amino acids with increasing concentrations of formaldehyde indicate the formation of dimethylol derivatives

$$CH_2O + H_2N-CHR-CO_2^- \rightleftarrows CH_2OH-NH-CHR-CO_2^-$$

$$CH_2O + CH_2OH-NH-CHR-CO_2^- \rightleftarrows (CH_2OH)_2N-CHR-CO_2^-$$

in which the basic properties of the nitrogen are suppressed to the point at which they are no longer discernible in the titration. Proline, which is incapable of forming a Schiff base or a dimethylol derivative, exhibits appreciable basic dissociation in the presence of even a large excess of formaldehyde.

When heated in acid solution with formaldehyde, amino acids [with the exception of glycine, which is converted into methylene diglycine,

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[67] Kapfhammer and Müller, Z. physiol. Chem., **225**, 1 (1934).
[68] Sörensen, Biochem. Z., **7**, 45 (1907).
[69] Franzen and Fellmer, J. prakt. Chem., [2] **95**, 299 (1917); Krause, Ber., **51**, 136, 542, 1556 (1918); **52**, 1211 (1919).
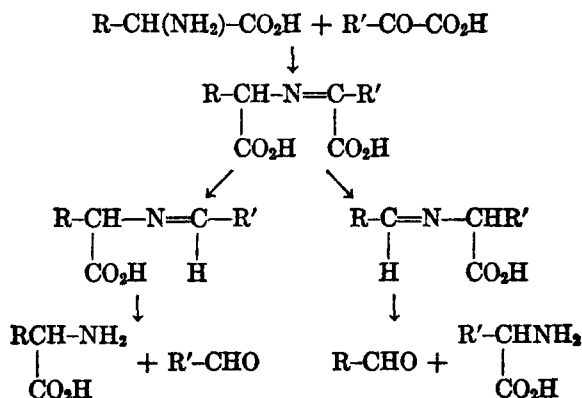[70] Tomiyama, J. Biol. Chem., **111**, 51 (1935).
[71] Levy, ibid., **99**, 767 (1933).

$CH_2(NH-CH_2-CO_2H)_2]$ [72] undergo extensive decomposition, a large proportion of their nitrogen being liberated in the form of methylamine.[73] It appears probable that this decomposition involves the transposition of the double bond of a Schiff base.[74]

$$CH_2=N-CHR-CO_2H \rightarrow CH_3N=CR-CO_2H \rightarrow CH_3NH_2 + R-CO-CO_2H$$
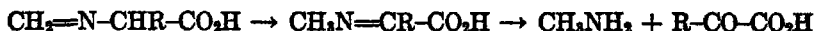
Similar decompositions are brought about by o-quinones,[75] methyl-glyoxal,[76] sugars,[77] isatin,[78] and α-keto acids.[79] In the last instance, the condensation product undergoes rearrangement with simultaneous decarboxylation, followed by hydrolysis. These reactions may proceed in two directions, with formation of the aldehyde derived either from the amino acid or from the keto acid, or both. When the former aldehyde is produced, a new amino acid is also formed.

$$R-CH(NH_2)-CO_2H + R'-CO-CO_2H$$
$$\downarrow$$
$$\underset{\underset{CO_2H}{|}}{R-CH}-N=\underset{\underset{CO_2H}{|}}{C}-R'$$

$$\underset{\underset{CO_2H}{|}}{R-CH}-N=\underset{\underset{H}{|}}{C}-R' \qquad \underset{\underset{H}{|}}{R-C}=N-\underset{\underset{CO_2H}{|}}{CHR'}$$
$$\downarrow \qquad\qquad \downarrow$$
$$\underset{\underset{CO_2H}{|}}{RCH-NH_2} + R'-CHO \qquad R-CHO + \underset{\underset{CO_2H}{|}}{R'-CHNH_2}$$

The nature of the substituents R and R' appears to determine which of the two carboxyl groups is eliminated.

Aromatic aldehydes yield condensation products with amino acids in the presence of alkali,[80] yielding Schiff bases $Ar-CH=N-CHR-CO_2Na$

[72] Löb, *Biochem. Z.*, **51**, 116 (1913).

[73] Zeleny and Gortner, *J. Biol. Chem.*, **90**, 427 (1931).

[74] Clarke, Gillespie, and Weisshaus, *J. Am. Chem. Soc.*, **55**, 4571 (1933).

[75] Kisch and collaborators, *Biochem. Z.*, **242**, 1 (1931); **244**, 440; **247**, 371; **249**, 63 (1932).

[76] Kisch, *ibid.*, **257**, 334 (1933).

[77] Akabori, *Ber.*, **66**, 143 (1933).

[78] Franke, *Biochem. Z.*, **258**, 296 (1933).

[79] Herbst and Engel, *J. Biol. Chem.*, **107**, 505 (1934); Herbst, *J. Am. Chem. Soc.*, **58**, 2239 (1936).

[80] Gerngross, *Biochem. Z.*, **108**, 89 (1920); Gerngross and Zuhlke, *Ber.*, **57**, 1462 (1924); Bergmann and collaborators, *Ber.*, **58**, 1034 (1925); *Z. physiol. Chem.*, **152**, 282 (1926); **172**, 277 (1927).

in which the double bond, being conjugated with the aromatic nucleus, shows less tendency to migrate and thereby to initiate further decomposition of the kind observed with aliphatic aldehydes. Subsequent condensations may, however, take place with further quantities of the aromatic aldehyde;[51] glycine and benzaldehyde yield N-benzylidene phenyl serine,

$$C_6H_5CH\!=\!N\!-\!CH\!-\!CHOH\!-\!C_6H_5$$
$$| $$
$$CO_2H$$
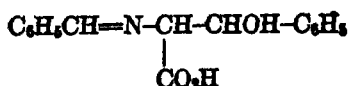
together with a by-product in which the carbon structure of the amino acid does not reappear.

$$C_6H_5CH\!=\!N\!-\!CH\!-\!CHOH\!-\!C_6H_5$$
$$| $$
$$C_6H_5$$
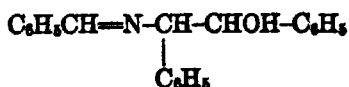
Measurements of optical activity [52] indicate that under milder conditions, in cold aqueous alcohol at pH 9–10, reversible equilibrium reactions take place between aromatic aldehydes and amino acids, with formation of compounds such as

$$Ar\!-\!CH(NH\!-\!CHR\!-\!CO_2H)_2$$

$$Ar\!-\!CHOH\!-\!NH\!-\!CHR\!-\!CO_2H$$

$$(Ar\!-\!CHOH)_2N\!-\!CHR\!-\!CO_2H$$
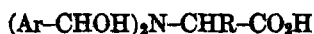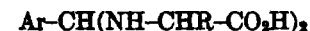
The maximum change of rotation is usually reached with 2–3 moles of the aldehyde.

The action of β-naphthoquinone and the closely related 1,2-naphthoquinone-4-sulfonic acid upon α-amino acids is of practical as well as theoretical interest. With aniline, both these compounds are converted into 2-hydroxy-1-naphthoquinone-4-anil, a red substance which resists the reducing action of sulfurous acid. In the first case,[53] part of the quinone is reduced to the hydroquinone;
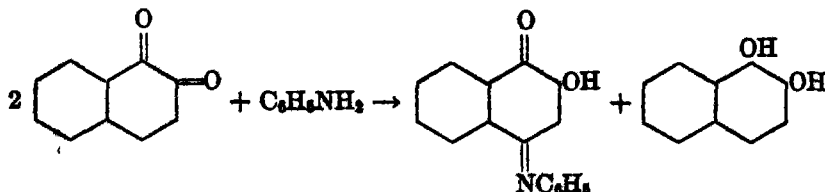
[51] Erlenmeyer and collaborators, *Ber.*, **25**, 3445 (1892); **28**, 1866 (1895); **30**, 1527, 2896 (1897); *Ann.*, **284**, 36 (1894); **307**, 79, 113 (1899); **337**, 205 (1904).

[52] Gulland and Mead, *J. Chem. Soc.*, **210** (1935).

[53] Liebermann, *Ber.*, **14**, 1310 (1881); Zincke, *Ber.*, **14**, 1493 (1881); Liebermann and Jacobson, *Ann.*, **211**, 36 (1882).

in the second,[84] the sulfonic group is eliminated as sulfur dioxide.

$$+ C_6H_5NH_2 \rightarrow \qquad + SO_2 + H_2O$$
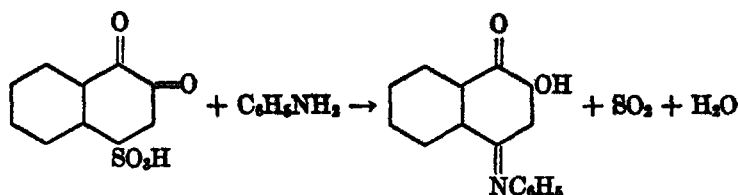
Amino acids appear to act similarly, yielding red solutions, the color of which, in contrast to that of the quinone reagent, is not discharged by thiosulfate.[85] The intensity of the color developed with β-naphthoquinonesulfonic acid is, with almost all the natural amino acids, proportional to their molecular concentration, so that the test can be applied for the quantitative estimation of amino acids in general.

The "ninhydrin" reaction, a sensitive color test in which a blue color is developed on warming amino acids with triketohydrindene hydrate in dilute aqueous solution, involves oxidative deamination.[86] The first step consists in the dehydrogenation of the amino acid, which passes over into ammonia and an aldehyde.

$$C(OH)_2 + \overset{R-CH-CO_2H}{\underset{NH_2}{\vert}} \rightarrow \qquad CHOH + RCHO + NH_3 + CO_2$$
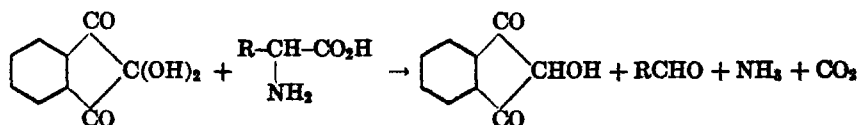
Triketohydrindene and its reduction product then condense with ammonia to yield the blue coloring matter,
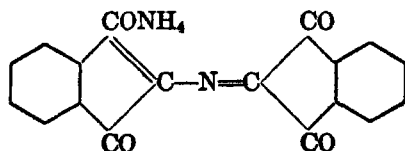
the constitution of which is analogous to that of murexide. Identical color intensities are developed with equimolar solutions of all α-amino acids and other compounds, such as dipeptides and aminoacetone, which contain α-aminoacyl groups.[87] Proline and hydroxyproline yield with triketohydrindene a different type of condensation product, in which only

[84] Böniger, Ber., 27, 23 (1894).
[85] Folin, J. Biol. Chem., 51, 377, 393 (1922).
[86] Ruhemann, J. Chem. Soc., 97, 2025 (1910); 99, 792, 1486 (1911); Abderhalden and Schmidt, Z. physiol. Chem., 72, 37 (1911); Harding and Warneford, J. Biol. Chem., 25, 319 (1916); Retinger, J. Am. Chem. Soc., 39, 1059 (1917).
[87] Cherbulies and Herzenstein, Helv. Chim. Acta, 17, 1440 (1934).

the carboxyl group has been eliminated.[88] That from proline has the constitution
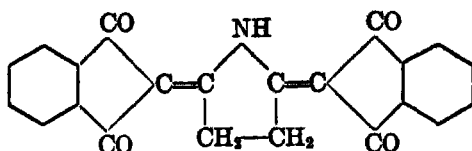
and possesses a red color.

The reaction with triketohydrindene has been applied to the estimation of α-amino acids [89] and some monoalkylamino acids [90] by measuring the amount of carbon dioxide evolved. Urea, peptides, esters, and amides of amino acids, acylamino acids, and N-dialkylamino acids do not react under the conditions specified.

Amino acids and esters, like simple amines, are converted by sodium hypochlorite into N-chloro derivatives, the process being almost independent of concentration.[91] The resulting products break down, slowly in the cold but rapidly on warming, into ammonia, carbon dioxide, and an aldehyde.

$$NH_2\text{-}CHR\text{-}CO_2H \rightarrow NHCl\text{-}CHR\text{-}CO_2H \rightarrow NH\text{=}CHR + CO_2$$

$$RCH\text{=}NH + H_2O \rightarrow RCHO + NH_3$$
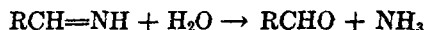
The reaction proceeds similarly with secondary amino acids; sarcosine yields methylamine in place of ammonia, proline breaks down into carbon dioxide and pyrroline.
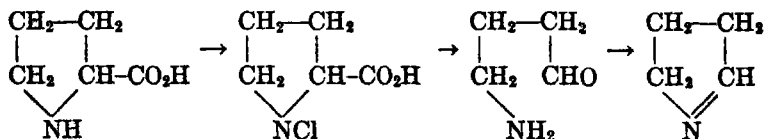
The presence of two alkyl groups or of an acyl group on the nitrogen atom inhibits the action of hypochlorite. Amino acids completely substituted in the α-position (e.g., α-aminoisobutyric acid) are, on the other hand, readily oxidized to ketones.
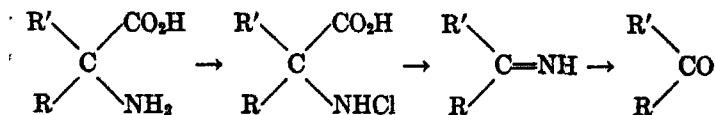
[88] Grassmann and v. Arnim, Ann., 509, 288 (1934).
[89] Van Slyke and collaborators, J. Biol. Chem., 141, 627, 671 (1941).
[90] Mason, Biochem. J., 32, 719 (1938).
[91] Langheld, Ber., 42, 2360 (1909).

Chloramine T (sodium p-toluenesulfonchloroamide) in neutral solution acts in the same way as hypochlorite,[92] yielding aldehydes, carbon dioxide, and ammonia when equimolecular quantities of the reactants are employed. With two moles of chloramine T, on the other hand, a different reaction occurs [93] whereby nitriles are formed.

$$
\begin{array}{ccc}
\text{RCHNH}_2 & \text{RCHNCl}_2 & \\
| & \rightarrow \quad | & \rightarrow \text{RCN} + 2\text{HCl} + \text{CO}_2 \\
\text{CO}_2\text{H} & \text{CO}_2\text{H} &
\end{array}
$$
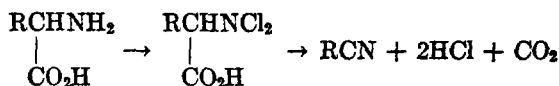
Both types of reaction occur when amino acids are oxidized with sodium hypobromite, the formation of aldehyde being favored, at the expense of nitrile production, by high alkalinity.[94]

Amino acids are oxidized by peroxides [95] or persulfates [96] with formation of aldehydes. Oxygen in the presence of charcoal, palladium black,[97] or finely divided iron [98] causes their breakdown to aldehydes or ketones. The fact tnat α-dimethylaminoisobutyric acid is readily oxidized by oxygen in the presence of charcoal,[99] yielding acetone, carbon dioxide, and dimethylamine, indicates that an addition compound with oxygen is formed. Ozone also breaks down not only the natural amino acids, but also dialkylamino acids, α-aminoisobutyric acid, and α-dimethylamino-isobutyric acid into aldehyde (or ketone), carbon dioxide, and volatile base. Similar products are formed from all the same types of compound by the action of silver oxide,[100] with which mono- and dimethylamino acids react more readily than the primary compounds; betaine and β-alanine are not attacked.

Amino acids are not oxidized by methylene blue alone, but are degraded to aldehydes by palladium black in the presence of a hydrogen acceptor such as alloxan or dinitrobenzene.[97] Charcoal is without action in the entire absence of oxygen.[101]

Amino acids are deaminated on exposure to ultra-violet light;[102] the reaction is specific for the α-amino grouping [103] and takes place in neutral, acid, or alkaline solution. Little is known of its mechanism.

[92] Dakin and collaborators, *Proc. Roy. Soc. (London)*, **B89**, 232 (1916); *Biochem. J.*, **11**, 79 (1917).

[93] Dakin, *Biochem. J.*, **10**, 319 (1916).

[94] Friedman and Morgulis, *J. Am. Chem. Soc.*, **58**, 909 (1936).

[95] Dakin, *J. Biol. Chem.*, **1**, 171 (1905); **4**, 63 (1908); **5**, 409 (1909).

[96] Lang, *Z. physiol. Chem.*, **241**, 68 (1936).

[97] Wieland and Bergel, *Ann.*, **439**, 196 (1924).

[98] Handovsky, *Z. physiol. Chem.*, **176**, 79 (1928).

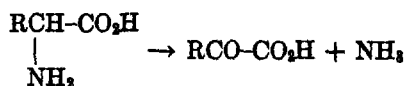[99] Bergel and Bolz, *ibid.*, **220**, 20 (1933).

[100] Herbst and Clarke, *J. Biol. Chem.*, **104**, 769 (1934).

[101] Wieland, Drishaus, and Koschara, *Ann.*, **513**, 203 (1934).
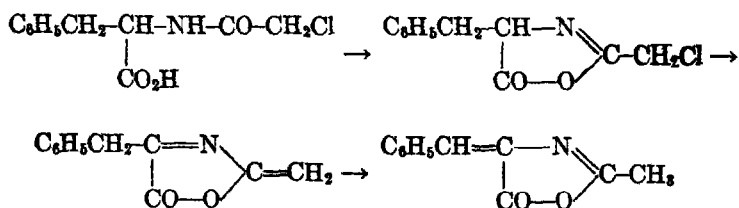
[102] Neuberg, *Biochem. Z.*, **13**, 305 (1908).

[103] Lieben and Urban, *ibid.*, **239**, 250 (1931).

The oxidative deamination of amino acids by biological systems, an important vital process, has been shown [104] to be effected by two enzyme systems present in kidney and liver, namely *l*-deaminase and *d*-deaminase, which respectively convert the natural and the unnatural varieties into ammonia and the corresponding α-keto acids,

$$\begin{array}{c} \text{RCH–CO}_2\text{H} \\ | \qquad\qquad \rightarrow \text{RCO–CO}_2\text{H} + \text{NH}_3 \\ \text{NH}_2 \end{array}$$

a process which has been shown [105] to be biochemically reversible to the natural form.

Dehydrogenation of amino acids to derivatives of the corresponding unsaturated amino acids has been effected by means of a remarkable reaction discovered by Bergmann and Stern.[106] When chloroacetylphenylalanine is warmed with acetic anhydride, the product consists of α-acetaminocinnamic azlactone, identical with the product of the action of benzaldehyde and acetic anhydride upon acetylglycine.[107]

$$\text{C}_6\text{H}_5\text{CH}_2\text{–CH–NH–CO–CH}_2\text{Cl} \qquad\rightarrow\qquad \begin{array}{c}\text{C}_6\text{H}_5\text{CH}_2\text{–CH—N}\\ | \qquad\qquad \diagdown\\ \text{CO—O}\diagup\text{C–CH}_2\text{Cl}\rightarrow\end{array}$$
$$\begin{array}{c}\text{CO}_2\text{H}\end{array}$$

$$\begin{array}{c}\text{C}_6\text{H}_5\text{CH}_2\text{–C}═\text{N}\\ | \qquad\qquad \diagdown\\ \text{CO—O}\diagup\text{C}═\text{CH}_2\rightarrow\end{array} \qquad \begin{array}{c}\text{C}_6\text{H}_5\text{CH}═\text{C—N}\\ | \qquad\qquad \diagdown\\ \text{CO—O}\diagup\text{C–CH}_3\end{array}$$

In like manner, α-bromopropionylalanine is converted into the azlactone of α-propionaminoacrylic acid, which on hydrolysis readily yields pyruvic acid; a similar series of reactions is undergone by α-bromopropionylasparagine.[108]

The conversion of chloroacetamino acids to unsaturated azlactones proceeds even more readily in the presence of pyridine.[109] With pyridine alone a betaine hydrochloride is formed; when this is treated with a mixture of acetic anhydride and pyridine, it yields the azlactone, but with acetic anhydride alone, it is converted into a cyclic compound of a novel type.

[104] Krebs, *Z. physiol. Chem.*, **217**, 191; **218**, 157 (1933); *Biochem. J.*, **29**, 1620 (1935).
[105] Knoop, *Z. physiol. Chem.*, **67**, 489 (1910); Knoop and Kertess, *ibid.*, **71**, 252 (1911); Neber, *ibid.*, **234**, 83 (1935).
[106] Bergmann and Stern, *Ann.*, **448**, 20 (1926).
[107] Erlenmeyer and Früstück, *Ann.*, **284**, 36 (1895).
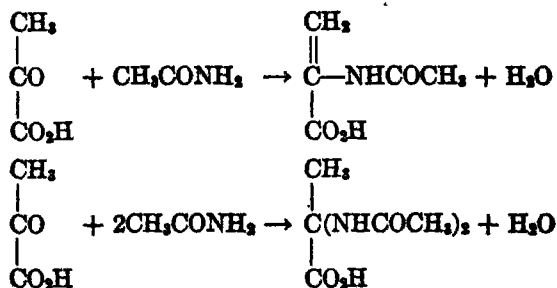[108] Bergmann, Kann, and Miekeley, *Ann.*, **449**, 135 (1926).
[109] Bergmann, Zervas, and Lebrecht, *Ber.*, **64**, 2315 (1931).

$$C_6H_5CH_2-CH-NHCOCH_2Cl$$
$$\underset{CO_2H}{|} \quad + C_5H_5N \rightarrow$$

$$C_6H_5CH_2-CH-NH-CO-CH_2-NC_5H_5$$
$$\underset{CO_2H}{|} \qquad\qquad \underset{Cl}{|}$$

$$\underset{Ao_2O + C_5H_5N}{\swarrow} \qquad\qquad \underset{Ao_2O}{\searrow}$$

$$C_6H_5CH=C-N\diagdown_{CCH_3}$$
$$\underset{CO-O}{|}$$

$$\underset{\qquad\qquad COCH_3}{}$$
$$C_6H_5CH_2CH-N-CO$$
$$\underset{C=C}{| \qquad |}$$
$$\underset{O^- \qquad N^+C_5H_5}{| \qquad |}$$
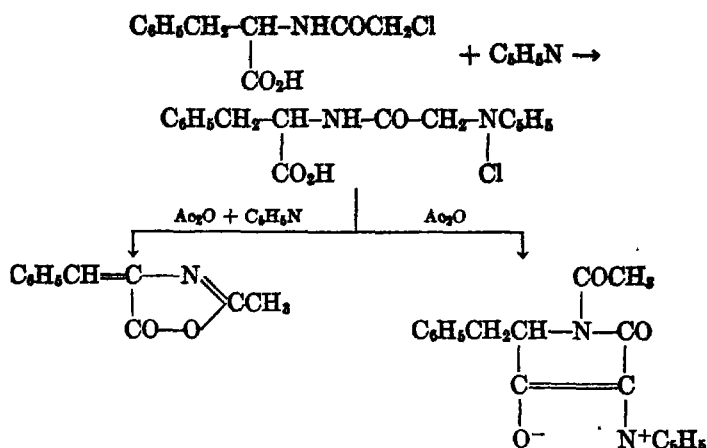
A similar product is formed by the action of a mixture of acetic anhydride and pyridine upon chloroacetyl glycine, which is of course incapable of yielding an unsaturated azlactone. These substances lose acetyl on heating with acid or alkali, but the pyridine can be regenerated only by pyrolysis or by disruptive oxidation.

$$\underset{NH-COCH_2Cl}{\overset{CH_2-CO_2H}{|}} \rightarrow \underset{NH-CO-CH_2-N^+C_5H_5}{\overset{CH_2-CO_2^-}{|}} \rightarrow \underset{CH_3CON-CO-C-N^+C_5H_5}{\overset{CH_2-C-O^-}{| \qquad ||}} \rightarrow$$

$$\underset{NH-CO-C-N^+C_5H_5}{\overset{CH_2-C-O^-}{| \qquad ||}} \xrightarrow{H_2O_2} \underset{NH-CO-CO_2H}{\overset{CH_2-CO_2H}{|}} + C_5H_5N$$
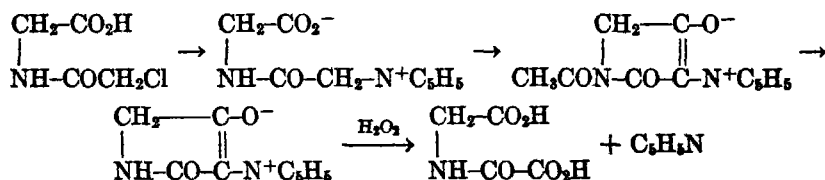
α,β-Unsaturated amino acids *per se* are apparently incapable of independent existence, but the presence of an acyl group on the nitrogen atom imparts sufficient stability to the molecule. A reversal of the effect of hydrolysis, mentioned above, can be brought about by condensing α-keto acids with acid amides. When, for instance, pyruvic acid is heated with acetamide, a mixture of α-acetaminoacrylic acid and α,α-diacetaminopropionic acid results.[110]

$$\underset{CO_2H}{\overset{CH_3}{\underset{|}{\overset{|}{CO}}}} + CH_3CONH_2 \rightarrow \underset{CO_2H}{\overset{CH_2}{\underset{|}{\overset{||}{C-NHCOCH_3}}}} + H_2O$$

$$\underset{CO_2H}{\overset{CH_3}{\underset{|}{\overset{|}{CO}}}} + 2CH_3CONH_2 \rightarrow \underset{CO_2H}{\overset{CH_3}{\underset{|}{\overset{|}{C(NHCOCH_3)_2}}}} + H_2O$$

[110] Bergmann and Grafe, *Z. physiol. Chem.*, **187**, 187, 196 (1930).

The diacetamino compound is readily converted into acetaminoacrylic acid by heating with acetic acid. With acetic anhydride it yields an azlactone,

$$CH_3CONH-\underset{\underset{CO-O}{|}}{\overset{\overset{CH_3}{|}}{C}}-N\diagdown_{C-CH_3}$$
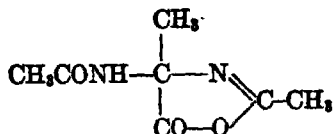
which readily reacts with amino acids to yield condensation products of peptide character.

$$CH_3CONH-\underset{\underset{CO-NH-CHR-CO_2H}{|}}{\overset{\overset{CH_3}{|}}{C}}-NHCOCH_3$$
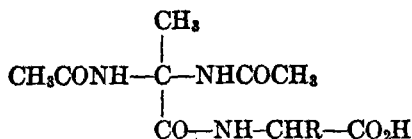
Acetaminoacrylic acid takes up hydrogen in presence of palladium, yielding dl-acetylalanine.

When an α-amino acid is digested in weakly acid solution with p-nitrophenylhydrazine, the bisnitrophenylhydrazone of the corresponding α-ketoaldehyde is gradually deposited.[111]

$$\underset{\underset{CO_2H}{|}}{RCHNH_2} \rightarrow \underset{\underset{CH=N-NH-C_6H_4NO_2}{|}}{RC=N-NH-C_6H_4NO_2} + NH_3$$
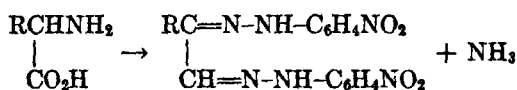
This remarkable reaction takes place not only with α-amino acids in general but also with α-hydroxy acids. It is of peculiar interest to the biochemist inasmuch as it constitutes a reversal of a biological synthesis of amino acids [112] in which a mixture of an α-ketonic aldehyde and ammonium carbonate, when perfused through a dog's liver, is converted into the corresponding amino acid. The reaction with α-hydroxy acids is likewise a reversal of the well-recognized enzymatic conversion of methylglyoxal to lactic acid.

GENERAL SYNTHETIC METHODS FOR PREPARING α-AMINO ACIDS

The classical method consisting in the action of ammonia upon α-halogen-substituted acids,

$$R-CHBr-CO_2H + 2NH_3 \rightarrow R-CH(NH_2)-CO_2H + NH_4Br$$

an example of which is the preparation of glycine,[113] has found the widest

[111] Dakin and Dudley, *J. Biol. Chem.*, **14**, 555; **15**, 127 (1913); Dakin, *Biochem. J.*, **10**, 313 (1916).

[112] Dakin and Dudley, *J. Biol. Chem.*, **18**, 29 (1914).

[113] Orten and Hill, *J. Am. Chem. Soc.*, **53**, 2797 (1931).

application. A modification of this principle, occasionally employed, involves the application of the phthalimide reaction [114] to esters of halogenated acids. The product is hydrolyzed with alkali, and the resulting acid heated with hydrochloric acid.

$$\begin{array}{c} RCHBr \\ | \\ CO_2Et \end{array} + KN\underset{CO}{\overset{CO}{<}}>C_6H_4 \longrightarrow \begin{array}{c} RCH-N \\ | \\ CO_2Et \end{array}\underset{CO}{\overset{CO}{<}}>C_6H_4 \xrightarrow{NaOH}$$

$$\begin{array}{c} R-CH-NH-COC_6H_4CO_2H \\ | \\ CO_2H \end{array} \xrightarrow{HCl} \begin{array}{c} R-CHNH_2 \cdot HCl \\ | \\ CO_2H \end{array} + C_6H_4(CO_2H)_2$$
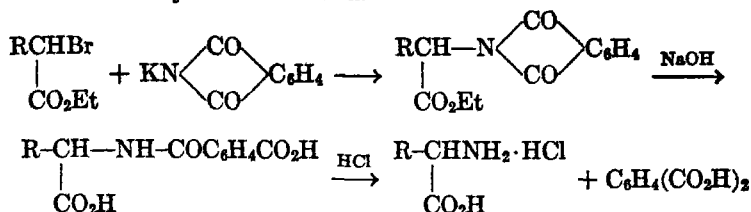
A general and extremely useful combination of the phthalimide and malonic ester synthesis has been developed by Sörensen.[115]

$$\begin{array}{c} CO_2Et \\ | \\ CHBr \\ | \\ CO_2Et \end{array} + KN\underset{CO}{\overset{CO}{<}}>C_6H_4 \rightarrow \begin{array}{c} CO_2Et \\ | \\ CHN \\ | \\ CO_2Et \end{array}\underset{CO}{\overset{CO}{<}}>C_6H_4$$

$$RBr + \begin{array}{c} CO_2Et \\ | \\ NaC-N \\ | \\ CO_2Et \end{array}\underset{CO}{\overset{CO}{<}}>C_6H_4 \rightarrow \begin{array}{c} CO_2Et \\ | \\ R-C-N \\ | \\ CO_2Et \end{array}\underset{CO}{\overset{CO}{<}}>C_6H_4 \xrightarrow[\text{heat}]{\text{hydrolysis}}$$

$$\begin{array}{c} RCHNH_2 \\ | \\ CO_2H \end{array} + C_6H_4(CO_2H)_2 + 2CO_2 + 2C_2H_5OH$$
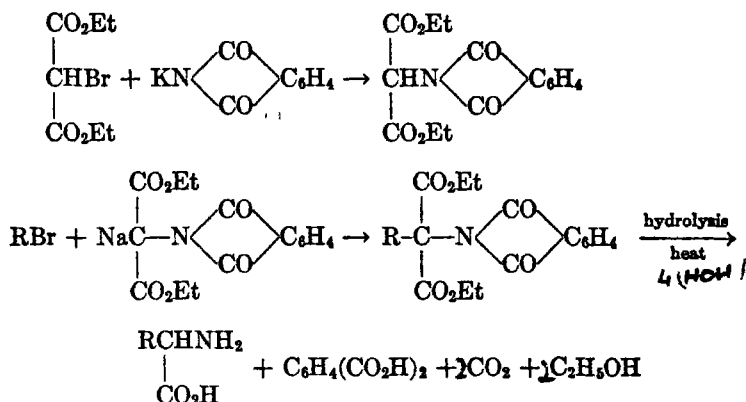
A variant of this procedure, in which the ethyl phthalimidomalonate is replaced by ethyl benzoylaminomalonate, has recently been proposed.[116, 117]

The Strecker synthesis [118] from aldehydes (or ketones), ammonia, and hydrogen cyanide is of wide application in a variety of forms, all of which involve the intermediate formation of aminonitriles: (1) by the interaction of a cyanohydrin and ammonia; [119]

$$RCHO \xrightarrow{HCN} \begin{array}{c} RCHOH \\ | \\ CN \end{array} \xrightarrow{NH_3} \begin{array}{c} R-CHNH_2 \\ | \\ CN \end{array} \xrightarrow{HCl} \begin{array}{c} RCHNH_2 \cdot HCl \\ | \\ CO_2H \end{array}$$
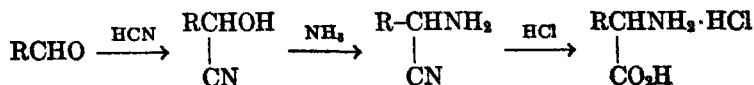
[114] Gabriel and Kroseberg, Ber., 22, 426 (1889).
[115] Sörensen, Z. physiol. Chem., 44, 448 (1905).
[116] Redemann and Dunn, J. Biol. Chem., 130, 341 (1939).
[117] Painter, J. Am. Chem. Soc., 62, 232 (1940).
[118] Strecker, Ann., 75, 27 (1850); Tiemann, Ber., 13, 381 (1880); 14, 1965 (1881).
[119] Menge, J. Am. Chem. Soc., 56, 2197 (1934).

(2) by the action of hydrogen cyanide on an aldehyde-ammonia;

$$RCHO \xrightarrow{NH_2} \overset{RCHOH}{\underset{NH_2}{|}} \xrightarrow{HCN} \overset{RCH-CN}{\underset{NH_2}{|}} \xrightarrow{HCl} \overset{RCH-CO_2H}{\underset{NH_2 \cdot HCl}{|}}$$
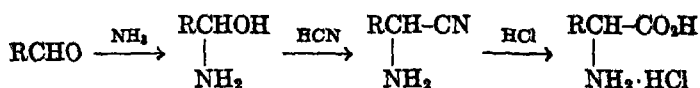
or (3) by treating an aldehyde with ammonium cyanide, produced either by direct union of ammonia and hydrogen cyanide or by mixing concentrated solutions of potassium cyanide and ammonium chloride.

Modifications of the Strecker process, whereby hydantoins are formed directly by the use of ammonium carbonate, have recently been developed.[120] Hydantoins, which break down to amino acids on hydrolysis under vigorous conditions, can, owing to their lack of dipolar properties, often be more readily isolated than the corresponding amino acids.

α-Amino acids have also been prepared by the reduction of α-oximino acids.[121] A general and more convenient method consists in the catalytic hydrogenation of α-keto acids in presence of ammonia,[122] a procedure of

$$\overset{O}{\underset{}{\overset{||}{R-C-COOH}}} + NH_3 + 2H \rightarrow \overset{NH_2}{\underset{}{\overset{|}{R-CH-COOH}}}$$
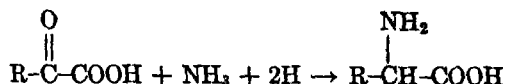
special value for the synthesis of amino acids with isotopic nitrogen.[123]

Of interest in this connection is the formation of acetylalanine by the action of ammonia upon pyruvic acid. [124]

$$2CH_3-CO-CO_2H + NH_3 \rightarrow \overset{CH_3-COH-CO_2H}{\underset{CH_3-COH-CO_2H}{\overset{|}{NH}}} \rightarrow \overset{CH_3-C-CO_2H}{\underset{CH_3-COH-CO_2H}{\overset{||}{N}}} \rightarrow$$

$$\overset{CH_3-CH-CO_2H}{\underset{CH_3-COH}{\overset{|}{\underset{}{\overset{N}{\underset{||}{}}}}}} + CO_2 \rightarrow \overset{CH_3-CH-CO_2H}{\underset{CH_3-CO}{\overset{|}{NH}}}$$
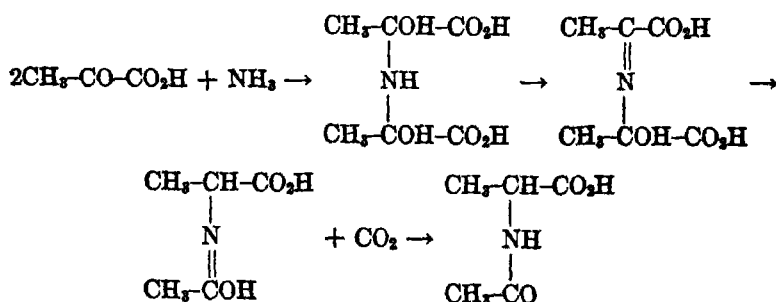
The decomposition of acid azides by alcohol, whereby urethanes are formed,

$$RCON_3 + EtOH \rightarrow RNHCO_2Et + N_2$$
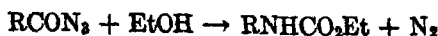
[120] Bucherer and collaborators, *J. prakt. Chem.*, [2] **140**, 291; [2] **141**, 5 (1934); Slotta, Behnisch, and Szyazka, *Ber.*, **67**, 1529 (1934).

[121] Gutknecht, *Ber.*, **13**, 1116 (1880).

[122] Knoop and Oesterlin, *Z. physiol. Chem.*, **148**, 294 (1925).

[123] Schoenheimer and Ratner, *J. Biol. Chem.*, **127**, 301 (1939).

[124] de Jong, *Rec. trav. chim.*, **19**, 259 (1900); Erlenmeyer, *Ann.*, **337**, 205 (1904).

has been applied [125] to the preparation of α-amino acids from malonic esters;

$$R-CH \begin{cases} CO_2Et \\ CO_2Et \end{cases} \rightarrow R-CH \begin{cases} CO_2Et \\ CO_2K \end{cases} \xrightarrow{N_2H_4} R-CH \begin{cases} CO-NH-NH_2 \\ CO_2K \end{cases} \xrightarrow{HNO_2}$$

$$R-CH \begin{cases} CON_3 \\ CO_2H \end{cases} \xrightarrow{EtOH} R-CH \begin{cases} NH-CO_2Et \\ CO_2H \end{cases} \xrightarrow{HCl} R-CH \begin{cases} NH_2 \\ CO_2H \end{cases}$$
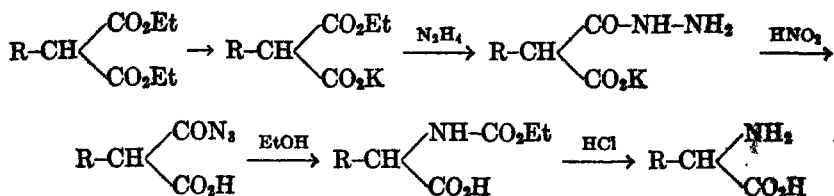
and from cyanoacetic esters. [126]

$$R-CH \begin{cases} CO_2Et \\ CN \end{cases} \rightarrow R-CH \begin{cases} CON_3 \\ CN \end{cases} \rightarrow R-CH \begin{cases} NHCO_2Et \\ CN \end{cases} \rightarrow R-CH \begin{cases} NH_2 \\ CO_2H \end{cases}$$
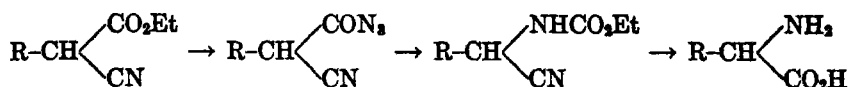
In syntheses of higher or more complex amino acids, particularly those containing a terminal aromatic group, a derivative of glycine has in many instances been employed as starting material. Benzaldehyde may be condensed with hippuric acid in presence of acetic anhydride [127] as in Perkin's synthesis, yielding the azlactone of benzoyl-α-amino-cinnamic acid, which on mild hydrolysis, followed by hydrogenation, yields benzoylphenylalanine.

$$C_6H_5CHO + \begin{matrix} CH_2-NHCOC_6H_5 \\ | \\ CO_2H \end{matrix} \rightarrow \begin{matrix} C_6H_5CH=C-N \\ | \quad\quad\quad\backslash CC_6H_5 \\ CO-O \end{matrix} \rightarrow$$

$$\begin{matrix} C_6H_5CH=C-NHCOC_6H_5 \\ | \\ CO_2H \end{matrix} \rightarrow \begin{matrix} C_6H_5CH_2-CH-NHCOC_6H_5 \\ | \\ CO_2H \end{matrix}$$
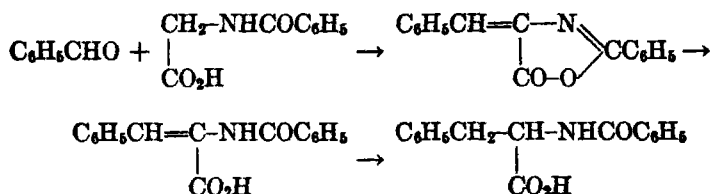
This is readily split by acid or by alkaline hydrolysis into dl-phenylalanine and benzoic acid. The benzoylaminocinnamic acid, on being subjected to drastic hydrolysis, breaks down into phenylpyruvic acid,

$$\begin{matrix} C_6H_5CH=C-NHCOC_6H_5 \\ | \\ CO_2H \end{matrix} \rightarrow C_6H_5CH_2-CO-CO_2H + C_6H_5CONH_2$$
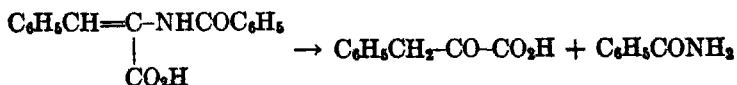
the oxime of which, on reduction, also yields phenylalanine. [128] Acetyl-glycine or even glycine itself may conveniently be employed. [106, 107, 129]

[125] Curtius, J. prakt. Chem., [2] 125, 211 (1930).
[126] Darapsky, ibid., [2] 146, 250 (1936).
[127] Erlenmeyer, Ann., 275, 1 (1893).
[128] Erlenmeyer, Ann., 271, 137 (1892).
[129] Dakin, J. Biol. Chem., 82, 439 (1929).

Similar syntheses have been carried out with hydantoin.[130]

$$\text{C}_6\text{H}_5\text{CHO} + \begin{array}{c} \text{CH}_2\text{—NH} \\ | \qquad\qquad \\ \text{CO—NH} \end{array}\!\!\!\!\rangle\text{CO} \rightarrow \begin{array}{c} \text{C}_6\text{H}_5\text{CH}{=}\text{C}\text{—NH} \\ | \qquad\qquad \\ \text{CO—NH} \end{array}\!\!\!\!\rangle\text{CO} \rightarrow$$

$$\begin{array}{c} \text{C}_6\text{H}_5\text{CH}_2\text{CH—NH} \\ | \qquad\qquad\quad \\ \text{CO—NH} \end{array}\!\!\!\!\rangle\text{CO} \rightarrow \begin{array}{c} \text{C}_6\text{H}_5\text{CH}_2\text{CH-NH}_2 \\ | \qquad\qquad\quad \\ \text{CO}_2\text{H} \end{array} + 2\text{NH}_3 + \text{CO}_2$$
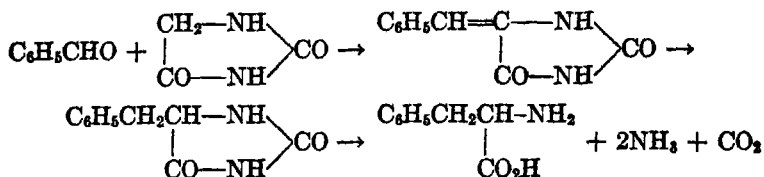
The last two steps (reduction and hydrolysis) can conveniently be carried out in one operation by treating the condensation product with ammonium sulfide at 58°.[131] Acetylthiohydantoin may advantageously be employed in place of hydantoin.

In this connection it may be noted that anisalhydantoin on alkaline hydrolysis yields, besides ammonia and p-methoxyphenylpyruvic acid, p-cresyl methyl ether and oxalic acid.

$$\begin{array}{c} \text{RCH}{=}\text{C}\text{—NH} \\ | \qquad\qquad \\ \text{CO—NH} \end{array}\!\!\!\!\rangle\text{CO} \rightarrow \begin{array}{c} \text{RCH}_2\text{—CO} \\ | \qquad\qquad \\ \text{CO}_2\text{H} \end{array}$$

$$\begin{array}{c} \text{RCH}{=}\text{C}\text{—NH} \\ | \qquad\qquad \\ \text{CO—NH} \end{array}\!\!\!\!\rangle\text{CO} \rightarrow \text{RCH}_3 + \begin{array}{c} \text{CO}_2\text{H} \\ | \\ \text{CO}_2\text{H} \end{array} + 2\text{NH}_3 + \text{CO}_2$$
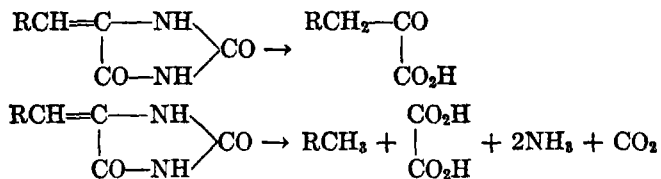
The last two are the principal products when concentrated alkalies are employed.[132]

Although the hydantoin method is most advantageously applicable to condensations with aromatic aldehydes, it can also be adapted to the synthesis of aliphatic amino acids. Heptaldehyde and hydantoin, when heated in acetic acid with sodium acetate, furnish a 15 per cent yield of heptylidene hydantoin, which on reduction with stannous chloride and subsequent alkaline hydrolysis is converted into α-aminopelargonic acid.[133]

An interesting synthesis involves the condensation of aromatic aldehydes with rhodanine.[134] The product on treatment with alkali breaks down to an α-thioketo acid, which with hydroxylamine yields the corresponding oximino acid; this is then reduced.

$$\text{C}_6\text{H}_5\text{CHO} + \begin{array}{c} \text{CH}_2\text{—S} \\ | \qquad\qquad \\ \text{CO—NH} \end{array}\!\!\!\!\rangle\text{CS} \rightarrow \begin{array}{c} \text{C}_6\text{H}_5\text{CH}{=}\text{C}\text{—S} \\ | \qquad\qquad \\ \text{CO—NH} \end{array}\!\!\!\!\rangle\text{CS} \rightarrow \begin{array}{c} \text{C}_6\text{H}_5\text{CH}_2\text{—CS} \\ | \\ \text{CO}_2\text{H} \end{array}$$

$$\rightarrow \begin{array}{c} \text{C}_6\text{H}_5\text{CH}_2\text{C}{=}\text{NOH} \\ | \\ \text{CO}_2\text{H} \end{array} \rightarrow \begin{array}{c} \text{C}_6\text{H}_5\text{CH}_2\text{CH-NH}_2 \\ | \\ \text{CO}_2\text{H} \end{array}$$
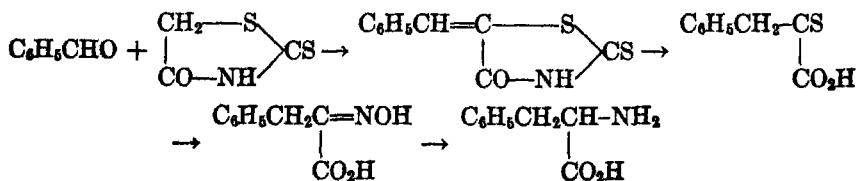
[130] Wheeler and Hoffman, Am. Chem. J., **45**, 368 (1911).

[131] Boyd and Robson, Biochem. J., **29**, 542, 546 (1935).

[132] Henze, Whitney, and Eppright, J. Am. Chem. Soc., **62**, 565 (1940).

[133] Johnson, ibid., **61**, 2485 (1939).

[134] Granacher, Helv. Chim. Acta, **5**, 610 (1922); **6**, 458 (1923).

All the above processes lead to inactive amino acids. Resolution has generally been effected by acylating the amino group and fractionally crystallizing the salts of the resulting acid with an optically active base such as an alkaloid. An alternative method recently developed [135] consists in introducing the *l*-menthoxyacetyl group and separating the resulting mixture of diastereoisomeric acids by crystallization. The "unnatural" (*d*) varieties of amino acids can be prepared by subjecting the racemic forms to the action of actively fermenting sugar solutions,[136] whereby amino acids having the *l* configuration are converted, by reductive deamination and decarboxylation, into alcohols,

$$R-CH(NH_2)-CO_2H \rightarrow R-CH_2OH + NH_3 + CO_2$$

the *d*-amino acids remaining intact.

An ingenious application of enzymatic reactions has been made [137, 138] to the resolution of racemic acylamino acids, which on treatment with aniline in the presence of the proteolytic enzyme papain yield the anilides of only the "natural" optical isomers. With, for example, the benzoyl and carbobenzoxy derivatives, only the *l* variety is converted into the anilide:

$$
\begin{array}{ccc}
R & & R \\
| & & | \\
H-C-NHCOC_6H_5 & \rightarrow & H-C-NHCOC_6H_5 \\
| & & | \\
CO_2H & & CONHC_6H_5
\end{array}
$$
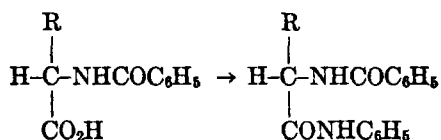
SOME INDIVIDUAL AMINO ACIDS AND DERIVATIVES

The subsequent pages contain discussion of the properties of all natural amino acids of protein origin except alanine, valine, leucine, isoleucine, norleucine, and phenylalanine. All these compounds are of great interest from the biochemical standpoint, but as they conform closely to the general type of monoamino monocarboxylic acid, reviewed above and treated in more detail in the case of glycine, their intimate discussion has not been undertaken.

Glycine. In addition to the general synthetic methods, outlined above, some special reactions have led to the formation of glycine. Cyanogen is simultaneously reduced and hydrolyzed by hot hydriodic acid.[139]

$$
\begin{array}{ccc}
CN & & CH_2NH_2 \\
| & + 5HI + 2H_2O \rightarrow & | \quad + NH_4I + 2I_2 \\
CN & & CO_2H
\end{array}
$$
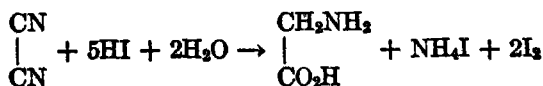
[135] Holmes and Adams, *J. Am. Chem. Soc.*, **56**, 2093 (1934).
[136] Ehrlich, *Biochem. Z.*, **1**, 8 (1906); **8**, 438 (1908).
[137] Bergmann and Fraenkel-Conrat, *J. Biol. Chem.*, **119**, 707 (1937).
[138] Fruton, Irving, and Bergmann, *ibid.*, **133**, 703 (1940).
[139] Emmerling, *Ber.*, **6**, 1351 (1873).

Hydrogen cyanide on long standing in the presence of moisture is converted into a crystalline polymer $C_3H_3N_3$ which on boiling with acids or alkalies breaks down into glycine.[140]

$$3HCN \rightarrow \begin{matrix} CN-CHNH_2 \\ | \\ CN \end{matrix} + 4H_2O \rightarrow \begin{matrix} CH_2NH_2 \\ | \\ CO_2H \end{matrix} + CO_2 + 2NH_3$$
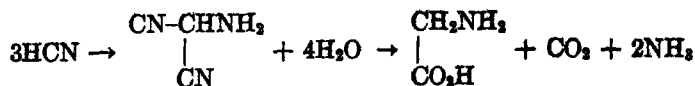
Glycine has been produced by treating malonic acid in concentrated sulfuric acid with hydrazoic acid.[141]

$$\begin{matrix} CO_2H \\ | \\ CH_2CO_2H \end{matrix} + HN_3 \rightarrow \begin{matrix} CON_3 \\ | \\ CH_2CO_2H \end{matrix} \rightarrow \begin{matrix} NCO \\ | \\ CH_2CO_2H \end{matrix} \rightarrow \begin{matrix} NH_2 \\ | \\ CH_2CO_2H \end{matrix}$$
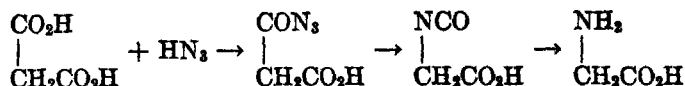
The detection and estimation of glycine in a protein hydrolysate have generally been effected by taking advantage of the sparing solubility of its ethyl ester hydrochloride [142] or of its picrate.[143] The selective precipitation of a complex potassium trioxalatochromiate, $[Cr(C_2O_4)_3]_6K_{18}(NH_2CH_2CO_2H)_5 \cdot 2H_2O$, has been suggested as a method for the quantitative isolation of glycine.[144] None of the other natural amino acids is precipitated under the conditions adopted. The analogous reagents in which the chromium is replaced by iron or cobalt are also selective precipitants for glycine. An almost equally specific precipitant is nitranilic acid (2,5-dinitro-3,6-dihydroxybenzoquinone), the glycine salt of which, $C_6O_2(OH)_2(NO_2)_2 \cdot 2CH_2(NH_2)CO_2H$, is sparingly soluble (0.8 per cent) in water and almost insoluble in alcohol.[145] No other amino acid, except histidine,[146] yields a salt of comparable solubility.

N-Benzoylglycine (hippuric acid) is excreted in the urine of mammals which receive benzoic acid by mouth. The benzoylation takes place in the kidney and, in some species, also in the liver.[147]

On treatment with cyanamide, glycine is converted into guanidinoacetic acid, or glycocyamine,

$$NH_2-CN + NH_2-CH_2-CO_2H \rightarrow NH_2-C(=NH)-NH-CH_2-CO_2H$$
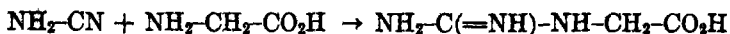
which is also formed by heating glycine with guanidine, or, more conveniently, S-methylisothiourea.[148]

$$NH_2-C(=NH)-SCH_3 + NH_2-CH_2-CO_2H \rightarrow NH_2-C(=NH)-NH-CH_2-CO_2H + CH_3SH$$
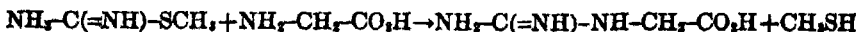
[140] Lange, Ber., 6, 99 (1873); Wippermann, Ber., 7, 767 (1874).

[141] Adamson, J. Chem. Soc., 1564 (1939).

[142] Fischer and Skita, Z. physiol. Chem., 33, 177 (1901); Fischer, ibid., 35, 227 (1902).

[143] Levene, J. Biol. Chem., 1, 413 (1906); Levene and Van Slyke, ibid., 12, 285 (1912).

[144] Bergmann and Fox, ibid., 109, 317 (1935).

[145] Town, Biochem. J., 30, 1833 (1936).

[146] Stein and Miller, J. Biol. Chem., 125, 599 (1938).

[147] Borsook and Dubnoff, ibid., 132, 307 (1939).

[148] Nencki and Sieber, J. prakt. Chem., [2] 17, 477 (1878); Wheeler and Merriam, Am. Chem. J., 29, 478 (1903).

On boiling with dilute hydrochloric acid, glycocyamine undergoes internal condensation to glycocyamidine.[149]

$$\begin{array}{ccc}
NH-CH_2-CO_2H & & NH-CH_2-CO \\
| & \rightarrow & | \quad\quad | \\
C(=NH)-NH_2 & & C(=NH)-NH
\end{array}$$
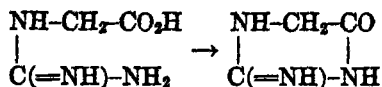
This compound is also formed by the action of guanidine upon glycine ester.[150]

$$NH_2-CH_2-CO_2Et + NH_2-C(=NH)-NH_2 \rightarrow$$

$$NH_2-CH_2-CO-NH-C(=NH)-NH_2 \rightarrow$$

$$\begin{array}{cc}
NH-CH_2-CO \\
| \quad\quad | \\
C(=NH)-NH
\end{array}$$
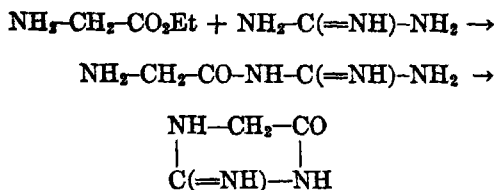
Sarcosine, the N-methyl derivative of glycine, has not been found among the hydrolytic products of proteins. It has been synthesized from ethyl chloroacetate and methylamine, by the action of formaldehyde and tin upon glycine in boiling acid,[72] and from benzenesulfonylglycine and methyl sulfate.[151] In its general properties it closely resembles glycine. On heating above 200° it breaks down, partly into carbon dioxide and dimethylamine and partly into water and the diketopiperazine, sarcosine anhydride.[152]

$$\begin{array}{cc}
CH_3N-CH_2-CO \\
| \quad\quad | \\
OC-CH_2-NCH_3
\end{array}$$
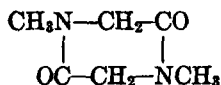
It is oxidized more rapidly than glycine by silver oxide (cf. p. 1101), yielding carbon dioxide, formaldehyde, and methylamine.

Creatine, an important constituent of muscle extract and of certain biological fluids, is α-methylguanidinoacetic acid; it has been synthesized from sarcosine and cyanamide,

$$\begin{array}{ccccc}
CH_2NHCH_3 & & CN & & CH_2-N(CH_3)-C=NH \\
| & + & | & \rightarrow & | \quad\quad\quad\quad | \\
CO_2H & & NH_2 & & CO_2H \quad\quad NH_2
\end{array}$$
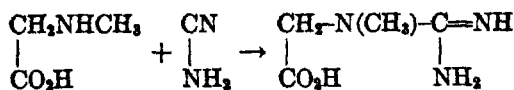
or (together with creatinine) from sarcosine and guanidine carbonate.[153] Its origin in the mammalian organism has been completely traced: the $N-CH_2CO_2H$ grouping is derived from glycine,[154] the ami-

[149] Jaffé, Z. physiol. Chem., 48, 430 (1906).
[150] Traube and Ascher, Ber., 46, 2077 (1913).
[151] Cocker and Lapworth, J. Chem. Soc., 1894 (1931).
[152] Mylius, Ber., 17, 286 (1884).
[153] Volhard, Jahresb., 685 (1868); Paulmann, Arch. Pharm., 232, 601 (1894).
[154] Bloch and Schoenheimer, J. Biol. Chem., 133, 633 (1940).

dine ($-C(=NH)-NH_2$) group comes from arginine,[155, 156] and the methyl from methionine.[157, 158]

Guanidine and its alkyl derivatives are in general very strong bases,[159] and creatine accordingly possesses dipolar properties which are even more pronounced than those of the amino acids.[160] From determinations of its dissociation constant in acid solution [161] and its solubility in alkaline solutions,[162] it has been estimated that creatine is a thousandfold stronger as a base than as an acid. Its solubility in cold water is 1.5 per cent; in solution it is neutral to litmus, and undoubtedly consists mainly of dipolar ions.

In boiling alkaline solution it breaks down into sarcosine, carbonic acid, and ammonia. The decomposition [163] proceeds along two different routes:

$$
\begin{array}{l}
CH_2-N(CH_3)-C=NH \\
\Big| \Big| \\
CO_2H NH_2
\end{array}
\nearrow
\begin{array}{l}
CH_2-NHCH_3 \\
\Big| \\
CO_2H \\
\searrow
\end{array}

\begin{array}{l}
CONH_2 \\
+\Big| \\
NH_2 \\

\end{array}
\searrow
\begin{array}{l}
CH_2NHCH_3 \\
\Big| \\
CO_2H \\
\nearrow
\end{array}
+ CO_2 + 2NH_3
$$

$$
\begin{array}{l}
CH-N(CH_3)-CO \\
\Big| \Big| \\
CO_2H NH_2
\end{array}
+ NH_3
$$
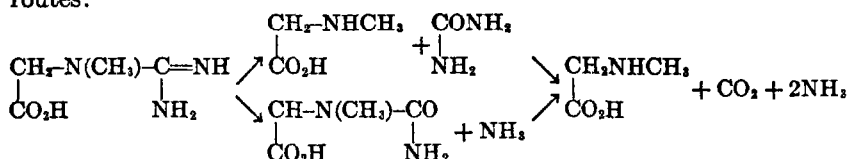
In acid solution, on the other hand, ring closure occurs, as in the hydantoic acids (p. 1095), with formation of creatinine.

$$
\begin{array}{l}
CH_2-N(CH_3)-C=NH \\
\Big| \Big| \\
CO_2H NH_2
\end{array}
\rightarrow
\begin{array}{l}
CH_2-N(CH_3)-C=NH \\
\Big| \Big| \\
CO\text{————}NH
\end{array}
+ H_2O
$$

        Creatine   Creatinine
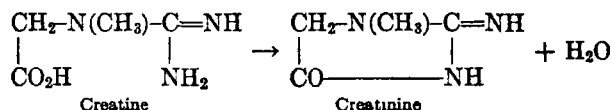
On treatment with alcoholic hydrogen chloride, creatine yields ester hydrochlorides.[164] The free esters, which should be extremely strong bases, appear to be incapable of independent existence, for when the hydrochloric acid is removed, alcohol is simultaneously split off, with formation of creatinine. This loss of alcohol also takes place merely on heating the hydrochloride alone or with water—so readily, indeed, that creatine ester hydrochlorides have been regarded as salts of creatinine in which alcohols are bound in some undetermined manner. However, titration

[155] Davenport, Fischer, and Wilhelmi, *Biochem. J.*, **32**, 262 (1938).
[156] Bloch and Schoenheimer, *J. Biol. Chem.*, **134**, 785 (1940).
[157] Borsook and Dubnoff, *ibid.*, **132**, 559 (1940).
[158] du Vigneaud, Chandler, Cohn, and Brown, *ibid.*, **134**, 787 (1940).
[159] Davis and Elderfield, *J. Am. Chem. Soc.*, **54**, 1499 (1932).
[160] Cannan and Shore, *Biochem. J.*, **22**, 920 (1928).
[161] Hahn and Barkan, *Z. Biol.*, **72**, 25 (1920); Eadie and Hunter, *J. Biol. Chem.*, **67**, 237 (1926).
[162] Hahn and Fasold, *Z. Biol.*, **82**, 473 (1925).
[163] Gaebler, *J. Biol. Chem.*, **69**, 613 (1926).
[164] Dox and Yoder, *ibid.*, **54**, 671 (1922); Kapfhammer, *Biochem. Z.*, **156**, 182 (1925) Failey and Brand, *J. Biol. Chem.*, **102**, 767 (1933).

curves of creatine ester salts not only clearly demonstrate the difference of the dissociation characteristics of the ester from those of creatinine but also indicate an irreversible conversion of ester to creatinine during the progress of the titration from pH 3.5 to pH 5.5. The readiness with which this ring closure takes place is not without parallel: on treating benzoylpseudoethylthiohydantoic ester with ammonia, the resulting ethyl ester of benzoylglycocyamine [165] spontaneously loses alcohol, at the moment of formation, to yield benzoylglycocyamidine.

$$
\begin{array}{ll}
CH_2\text{-NH-}C\text{=}NCOC_6H_5 \\
\quad | \qquad\quad | \\
CO_2Et \quad\ SEt
\end{array}
+ NH_3 \rightarrow
\begin{array}{ll}
CH_2\text{-NH-}C\text{=}NCOC_6H_5 \\
\quad | \qquad\quad | \\
CO_2Et \quad\ NH_2
\end{array}
\rightarrow
$$

$$
\begin{array}{ll}
CH_2\text{-NH-}C\text{=}NCOC_6H_5 \\
\quad | \qquad\quad | \\
CO\text{------}NH
\end{array}
$$
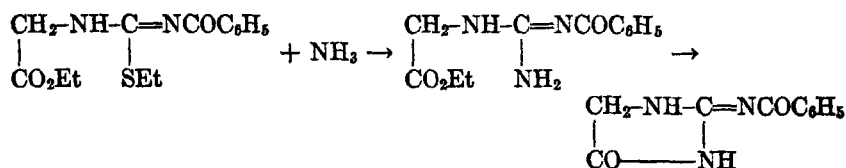
Analogous cases are cited on pp. 1117, 1145, and 1147.

On treatment with acetic anhydride, creatine is converted into a substance which is split by ammonia into acetylsarcosine amide and acetylurea; [166] the reaction is explained as involving the rearrangement of a quaternary ammonium acetate of acetyl creatinine:

$$
\begin{array}{l}
CH_2\text{---}NCH_3 \\
\ |\quad\ \ \rangle C\text{=}NH \\
CO_2H\ \ NH_2
\end{array}
\rightarrow
\begin{array}{l}
\quad\ CH_3CO\quad OCOCH_3 \\
\qquad\qquad\backslash\!/ \\
CH_2\text{-}NCH_3 \\
\ |\quad\ \rangle C\text{=}NH \\
CO\text{---}NH
\end{array}
\rightarrow
\begin{array}{l}
CH_2\text{-}NCH_3\text{-}COCH_3 \\
\ | \\
CO\text{-}NH\text{-}C\text{=}NH \\
\qquad\qquad | \\
\qquad\quad\ OCOCH_3
\end{array}
\rightarrow
$$

$$
\begin{array}{l}
CH_2\text{-}NCH_3\text{-}COCH_3 \\
\ | \\
CO\text{-}NHCONHCOCH_3
\end{array}
$$
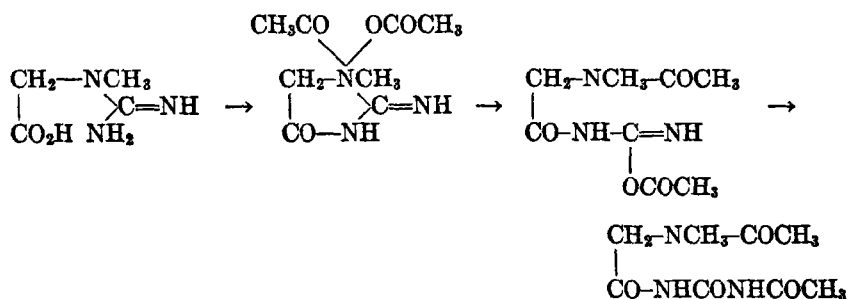
Of great biochemical interest is creatine-phosphoric acid, or phosphocreatine,

$$
\begin{array}{l}
CH_2\text{-}N(CH_3)\text{-}C\text{=}NH \\
\ | \qquad\qquad\quad | \\
CO_2H \qquad\quad NH\text{-}PO(OH)_2
\end{array}
$$
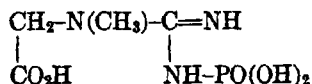
an unstable constituent of mammalian muscle tissue.[167] The hydrolytic breakdown of this substance into creatine and phosphoric acid, which occurs under the influence of enzymes present in muscle, is exothermic

[165] Johnson and Nicolet, *J. Am. Chem. Soc.*, **37**, 2416 (1915).

[166] Ing, *J. Chem. Soc.*, 2198 (1932).

[167] Eggleton and Eggleton, *Biochem. J.*, **21**, 190 (1927); Meyerhof and Lohmann, *Biochem. Z.*, **196**, 22, 49 (1928); Fiske and Subbarow, *J. Biol. Chem.*, **81**, 629 (1929); Parnas and Ostern, *Biochem. Z.*, **279**, 94 (1935).

and is associated with the development of muscular energy; regeneration occurs during repose.

Creatinine is more soluble in water and, being more electrically unbalanced, behaves as a stronger base than creatine.[161, 168] In aqueous solution, creatine and creatinine enter into equilibrium, the stationary state being established very slowly in the cold but more rapidly at higher temperatures. The reaction rate also depends on the pH level, reaching a maximum at pH 4.[160] The composition of the equilibrium mixture depends upon the pH of the solution, the ratio of creatinine to creatine rising rapidly from 2 at pH 4 to 20 at pH 2. At pH 5 to 7, the components are present in approximately equimolar ratio.[169] In more strongly alkaline solution, hydrolysis to ammonia, methylhydantoin, urea, and sarcosine occurs.[163]

Creatinine forms a characteristic picrate which is sparingly soluble in water. On the addition of alkali, this yellow picrate develops an orange-red color. Creatine also forms a picrate which closely resembles that of creatinine but yields merely a yellow solution on treatment with alkali. These observations, recorded by Jaffé, form the basis of an analytical method for the estimation of creatinine.[170] The development of the red color in this test is not specific for creatinine, and has been observed with glycocyamidine, hydantoins, barbituric acid, and diketopiperazines. On the other hand, no color is formed by derivatives of creatinine in which both of the imino hydrogen atoms are replaced by methylol groups, or the methylene hydrogen atoms by benzylidene. The red color was long supposed to be due to picramic acid, which is formed from picric acid by a variety of reducing substances; it now appears to be caused by the formation of a salt of a red tautomer of creatinine picrate.[171] Picric acid seems to be essential for the Jaffé reaction, which does not occur with 2,4- and 2,6-dinitrophenols or even with 2,4,6-trinitro-*m*-cresol.

Strains of bacteria have been discovered [172] which rapidly bring about the oxidative breakdown of creatine and creatinine, with formation of urea. They act similarly, but more slowly, with glycocyamidine and other substances which contain the grouping =N—C—N=.[173]

[168] McNally, *J. Am. Chem. Soc.*, **48**, 1003 (1926).
[169] Edgar and Shiver, *ibid.*, **47**, 1179 (1925).
[170] Jaffé, *Z. physiol. Chem.*, **10**, 391 (1886); Folin, *ibid.*, **41**, 223 (1904); *J. Biol. Chem.*, **17**, 463, 469, 475 (1914); Folin and Doisy, *ibid.*, **28**, 349 (1917).
[171] Greenwald and Gross, *ibid.*, **59**, 601 (1924). Greenwald, *J. Am. Chem. Soc.*, **47**, 1443 (1925); *J. Biol. Chem.*, **77**, 539; **80**, 103 (1928); **86**, 333 (1930); Anslow and King, *J. Chem. Soc.*, 1210 (1929).
[172] Dubos and Miller, *J. Biol. Chem.*, **121**, 429 (1937).
[173] Krebs and Eggleston, *Enzymologia*, **7**, 310 (1939).

Dimethylglycine can be prepared by the interaction of chloroacetic acid and dimethylamine; by condensing formaldehyde cyanohydrin with dimethylamine and then hydrolyzing; [174] also, by treating glycine with formaldehyde in the presence of tin and hydrochloric acid [72] or of formic acid.[74] The methyl ester, when heated, rearranges reversibly into betaine,

$$(CH_3)_2N-CH_2-CO_2CH_3 \rightleftarrows (CH_3)_3N^+-CH_2-CO_2^-$$
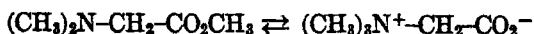
an example of the alkylating action of carboxylic esters.[175]

Betaine salts such as the hydrochloride,which can be titrated as monocarboxylic acids, are formed by the addition of chloroacetic acid to trimethylamine or by treating glycine or sarcosine with methyl iodide or sulfate. At 260–270° betaine hydrochloride breaks down into tetramethylammonium chloride and carbon dioxide.

Esters of betaine are of course capable of existing only in the form of salts; these are formed directly from trimethylamine and chloroacetic esters, from methyl iodide and an ester of dimethylglycine, or as a by-product in the methylation of glycine.[176] On treatment with ammonia they are converted into salts of betaine-amide. The corresponding hydrazide,[177] similarly produced from hydrazine, has recently found application as a reagent for preparing water-soluble derivatives of insoluble ketones.

Aspartic and Glutamic Acids. The chemical character of the mono-amino dicarboxylic acids resembles in the main that of the monocarboxylic acids; such differences as exist are ascribable to the presence of the second carboxyl group.

Both acids are less soluble in water than the corresponding mono-carboxylic acids; the pH of their aqueous solutions is low, but higher than the isoelectric point (cf. p. 1088). They are extracted by butyl alcohol from their solutions at pH 3.[23] Both form sparingly soluble salts with heavy metals; the salts of barium and calcium are insoluble in alcohol.[178] $l(+)$-Aspartic acid and $l(+)$-glutamic acid are both dextrorotatory in strongly acid solution and levorotatory at the isoelectric point; in alkaline solution the aspartic acid rotates to the left and the glutamic acid to the right.

dl-Aspartic acid is formed by direct addition of ammonia to maleic

[174] Eschweiler, Ann., **279**, 39 (1894).
[175] Willstätter and collaborators, Ber., **35**, 584, 2757 (1902); Hammett and Pfluger, J. Am. Chem. Soc., **55**, 4079 (1933).
[176] Novak, Ber., **45**, 834 (1912).
[177] Girard and Sandulesco, Helv. Chim. Acta, **19**, 1095 (1936).
[178] Foreman, Biochem. J., **8**, 461, 481 (1914).

or fumaric acid.[179] Ethyl fumarate reacts with ammonia to form diethyl aspartate [180] and diketopiperazine diacetamide,[181]

$$NH_2CO-CH_2-CH-CO-NH$$
$$NH-CO-CH-CH_2-CONH_2$$
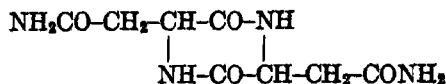
which on alkaline hydrolysis is converted into dl-aspartic acid.[182] Syntheses of glutamic acid have followed more conventional lines.

An important difference between aspartic and glutamic acids is represented by the readiness with which the latter passes over into pyrrolidonecarboxylic acid in hot aqueous solution.[178]

$$CH_2-CH_2-CH-CO_2H \qquad CH_2-CH_2-CH-CO_2H$$
$$CO_2H \quad NH_2 \rightarrow CO------NH \qquad + H_2O$$
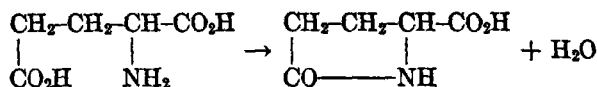
This reaction, which is reversed by the action of hot concentrated hydrochloric acid, finds no analogy in the case of aspartic acid, and is referable to the spatial proximity, in glutamic acid, of the groups involved.

Another difference, probably due to a similar cause, resides in the contrasting stabilities of the naturally occurring monoamides of the two acids, both of which are widely distributed in growing vegetation and have been isolated from enzymatic digests of vegetable proteins.[183] Asparagine, $NH_2COCH_2CH(NH_2)CO_2H$, is relatively stable in aqueous solution over a wide range of hydrogen-ion concentration. Glutamine, $NH_2COCH_2CH_2CH(NH_2)CO_2H$, rapidly undergoes hydrolysis to ammonium pyrrolidonecarboxylate [184] in neutral solution at 100°, under which conditions asparagine is hardly affected. Introduction of an aminoacyl group into glutamine stabilizes the amide linkage, possibly by reducing the acidic dissociation of the neighboring carboxyl group.

l(+)-Glutamine, which is dextrorotatory in acid but levorotatory in water, appears to be intimately involved in the detoxification of ammonia and the assimilation of nitrogen by plants and animals. Vegetable organisms grown under conditions in which ammonia is the sole source of nitrogen accumulate relatively large proportions of glutamine,[185] which is also synthesized in kidney and other tissues from l(+)-glutamic acid and ammonia.[186]

[179] Engel, *Bull. soc. chim.*, [2] **48**, 97 (1887); **50**, 149 (1888).
[180] Koerner and Menozzi, *Gazz. chim. ital.*, **17**, 226 (1887).
[181] Fischer and Koenigs, *Ber.*, **37**, 4585 (1904).
[182] Dunn and Fox, *J. Biol. Chem.*, **101**, 493 (1933).
[183] Damodaran and collaborators, *Biochem. J.*, **26**, 235, 1704 (1932).
[184] Vickery and collaborators, *ibid.*, **29**, 2710 (1935).
[185] Greenhill and Chibnall, *ibid.*, **28**, 1422 (1934); Vickery and collaborators, *Science*, **80**, 459 (1934); *J. Biol. Chem.*, **113**, 157 (1936); *Plant Physiol.*, **11**, 413 (1936).
[186] Krebs, *Biochem. J.*, **29**, 1951 (1935).

The synthetic conversion of $l(+)$-glutamic acid into the natural variety of glutamine exemplifies some of the elegant preparative methods developed by Bergmann.[187] On treatment with benzyl chlorocarbonate, glutamic acid yields the carbobenzoxy derivative, which with acetic anhydride is transformed into an anhydride.

$$C_6H_5CH_2OCONH-CH-CH_2-CH_2 \qquad C_6H_5CH_2OCONH-CH-CH_2-CH_2$$
$$\qquad\qquad\quad | \qquad\quad | \qquad \rightarrow \qquad\qquad\qquad | \qquad\quad |$$
$$\qquad\qquad COOH \quad COOH \qquad\qquad\qquad\qquad CO-O-CO$$
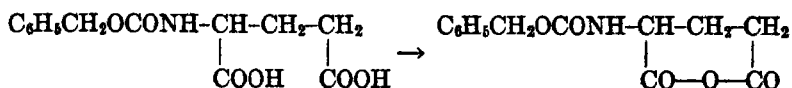
This on treatment with ammonia yields a carbobenzoxy derivative of isoglutamine,[187]

$$CH_2-CH_2-CH-NHCO_2CH_2C_6H_5$$
$$\quad | \qquad\qquad |$$
$$CO_2H \qquad CONH_2$$
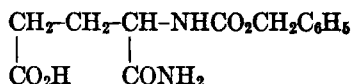
isomeric with that from natural glutamine. A similar reaction occurs with benzyl alcohol, the α-monobenzyl ester being the sole product. This is converted, through the chloride, into the amide; when this compound is catalytically hydrogenated, the benzyl groups are split off as toluene and the resulting unstable carbamic acid grouping loses carbon dioxide, leaving glutamine.

$$C_6H_5CH_2OCONH-CH-CH_2-CH_2 \qquad C_6H_5CH_2OCONH-CH-CH_2-CH_2$$
$$\qquad\qquad\quad | \qquad\quad | \qquad \rightarrow \qquad\qquad\qquad\quad | \qquad\qquad | \qquad \rightarrow$$
$$\qquad\qquad CO_2C_7H_7 \quad CO_2H \qquad\qquad\qquad\qquad CO_2C_7H_7 \qquad CONH_2$$

$$C_6H_5CH_3 + CO_2 \qquad NH_2-CH-CH_2-CH_2-CONH_2$$
$$\qquad\qquad + \qquad\qquad\qquad\qquad | \qquad\qquad\qquad\qquad + CH_3C_6H_5$$
$$\qquad\qquad\qquad\qquad\qquad\qquad CO_2H$$
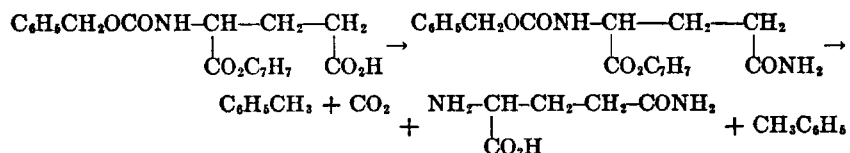
Isoglutamine is formed in the same way from its carbobenzoxy derivative. Aspartic acid may be converted into asparagine and isoasparagine by an analogous series of reactions.

The γ-monoethyl ester of glutamic acid, prepared by the action of ethyl iodide upon silver glutamate or by direct esterification of glutamic acid, readily undergoes auto-condensation to pyrrolidonecarboxylic acid.

$$CH_2-CH_2-CH-CO_2H \qquad CH_2-CH_2-CH-CO_2H$$
$$\quad | \qquad\qquad | \qquad \rightarrow \quad | \qquad\qquad |$$
$$CO_2Et \qquad NH_2 \qquad\qquad CO-----NH$$
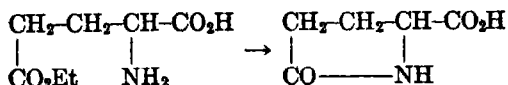
When its carbobenzoxy derivative is successively treated with ammonia and hydrogenated, glutamine results.[188]

[187] Bergmann and collaborators, Z. physiol. Chem., **221**, 51 (1933); Ber., **65**, 1192 (1932); **66**, 1288 (1933).

[188] Nienburg, Ber., **68**, 2232 (1935).

$$CH_2-CH_2-CH-CO_2H \qquad\qquad CH_2-CH_2-CH-CO_2H$$
$$|\qquad\qquad | \qquad\qquad\to \qquad | \qquad\qquad | \qquad\qquad\to$$
$$CO_2Et \qquad NH-CO_2CH_2C_6H_5 \qquad CONH_2 \quad NH-CO_2CH_2C_6H_5$$

$$CH_2-CH_2-CH-CO_2H$$
$$|\qquad\qquad |$$
$$CONH_2 \quad NH_2$$
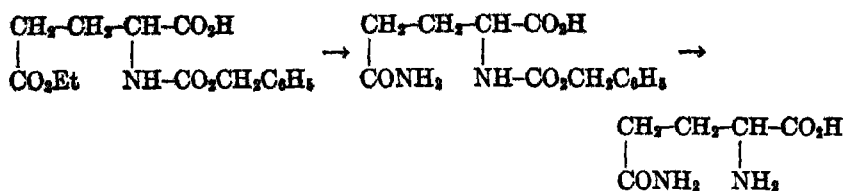
On treatment with hypobromite, followed by hydrolysis, *l*-asparagine is converted into an optically active α,β-diaminopropionic acid identical with that prepared by the action of ammonia upon the β-chloroalanine derived from natural serine.
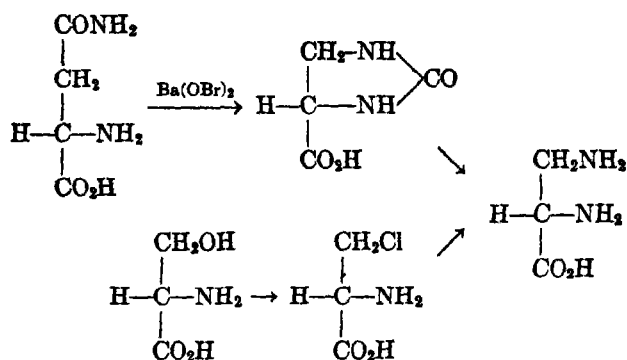
This series of reactions establishes the configurational identity of natural aspartic acid and serine. By a similar process, glutamine yields an α,γ-diaminobutyric acid which with its dibenzoyl esters displays optical relations analogous to those shown by the natural α,δ- and α,ϵ-diamino acids ornithine and lysine.[189] This *l*-α,γ-diaminobutyric acid is also formed [141] by the action of hydrazoic acid upon *l*-glutamic acid in concentrated sulfuric acid (*cf.* p. 1106).

Proline. The widely distributed *l*(−)-proline is distinguished from all other amino acids, except hydroxyproline, by its inability to yield nitrogen on treatment with nitrous acid and by its ready solubility in alcohol. For isolation, it may be precipitated [190] (after the removal of histidine and arginine) as the rhodanilate by means of ammonium rhodanilate, $[Cr(SCN)_4 \cdot (C_6H_5NH_2)_3]_2(NH_4)_2 \cdot 3H_2O$. Reinecke salt,[191] $[Cr(SCN)_4 \cdot (NH_3)_2]NH_4 \cdot H_2O$, forms a similar precipitate with both proline and hydroxyproline.[192]

[189] Karrer and collaborators, *Helv. Chim. Acta*, **6**, 411, 957 (1923); **9**, 301 (1926).
[190] Bergmann, *J. Biol. Chem.*, **110**, 471 (1935).
[191] Dakin, "Organic Syntheses," John Wiley & Sons, New York (1935), Vol. 15, p. 74.
[192] Kapfhammer and collaborators, *Z. physiol. Chem.*, **170**, 294 (1927); **173**, 245 (1928).

Proline has been synthesized by several methods. The most obvious, the hydrogenation of α-pyrrolecarboxylic acid, proceeds with difficulty, but can be accomplished in acid alcoholic solution by means of platinum oxide activated by ferric chloride.[193]

$$
\begin{array}{c}
\text{CH}\!-\!-\!-\!\text{CH} \\
\| \qquad \| \\
\text{CH-NH-C-CO}_2\text{H}
\end{array}
\rightarrow
\begin{array}{c}
\text{CH}_2\!-\!-\!-\!\text{CH}_2 \\
| \qquad | \\
\text{CH}_2\text{-NH-CH-CO}_2\text{H}
\end{array}
$$
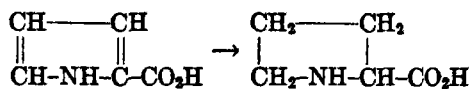
A more convenient process involves the hydrogenation, under high pressure in the presence of Raney nickel, of the 1,2-dicarbethoxypyrrole obtained by the action of ethylmagnesium bromide and ethyl chlorocarbonate upon pyrrole.[194]
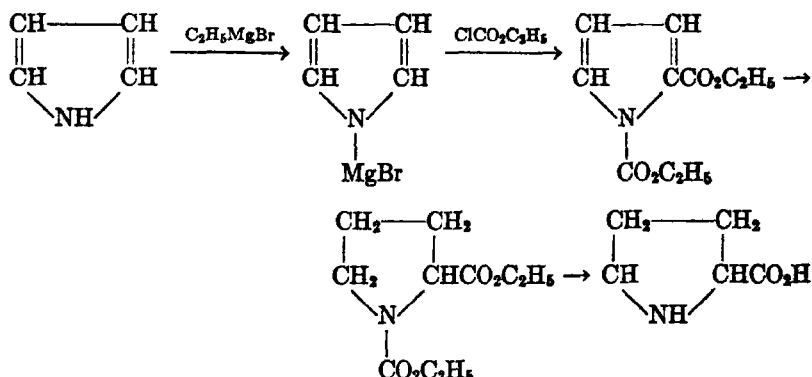
Pyrrolidonecarboxylic ester yields proline on reduction with sodium and alcohol.[195]

An interesting synthesis starts from α-piperidone.[196]
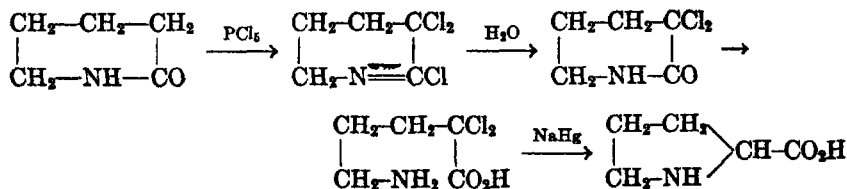
Like other amino acids, proline yields an ester hydrochloride on boiling with alcoholic hydrogen chloride. The free esters of proline are

[193] Putokhin, J. Russ. Phys.-Chem. Soc., 62, 2209 (1930) [C. A., 25, 3995 (1931)].
[194] Signaigo and Adkins, J. Am. Chem. Soc., 58, 1122 (1936).
[195] Fischer and Boehner, Ber., 44, 1332 (1911).
[196] Heymons, Ber., 66, 846 (1933).

unstable and spontaneously lose the elements of alcohol to yield the tricyclic diketopiperazine, proline anhydride.[197]

$$CH_2-CH_2-N-CO-CH-CH_2-CH_2$$
$$CH_2-\!\!-\!\!-\!\!-CH-CO-N-\!\!-\!\!-\!\!-CH_2$$
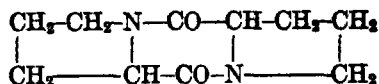
On methylation, proline is converted into its betaine, stachydrine, the picrate, mercurichloride, and aurichloride of which are sparingly soluble and afford a form in which proline can be quantitatively estimated.[198] Stachydrine, when heated under reduced pressure at 235°, passes over into the isomeric methyl ester of hygric acid.[199]

$$\begin{array}{c} CH_2-CH_2 \\ CH_2-CH \end{array}\!\!\!>\!\!\overset{+}{N}(CH_3)_2 \rightleftarrows \begin{array}{c} CH_2-CH_2 \\ CH_2-CH \end{array}\!\!\!>\!\!NCH_3$$
$$\quad\quad\ CO_2^- \quad\quad\quad\quad\quad CO_2CH_3$$
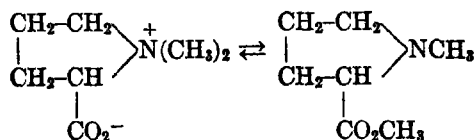
Serine. $l(+)$-Serine, which is dextrorotatory in acid solution but levorotatory in water, occurs in proteins of both animal and vegetable origin, and appears to be a constituent, together with ethanolamine, of cephalin.[200] It has been synthesized by applying the Strecker cyanhydrin procedure to glycollaldehyde and, more conveniently, to ethoxyacetaldehyde;[201] in the latter synthesis the final hydrolysis is effected with hydrobromic acid to remove the ethyl group. Serine has also been synthesized by the condensation of ethyl hippurate with ethyl formate, with subsequent reduction and hydrolysis,[202]

$$HCO_2Et + \begin{array}{c} NH-COC_6H_5 \\ CH_2-CO_2Et \end{array} \xrightarrow{EtONa} \begin{array}{c} NH-COC_6H_5 \\ HCO-CH-CO_2Et \end{array} \xrightarrow{NaHg}$$

$$\begin{array}{c} NH-COC_6H_5 \\ HOCH_2-CH-CO_2Et \end{array} \rightarrow \begin{array}{c} NH_2 \\ HOCH_2-CH-CO_2H \end{array}$$
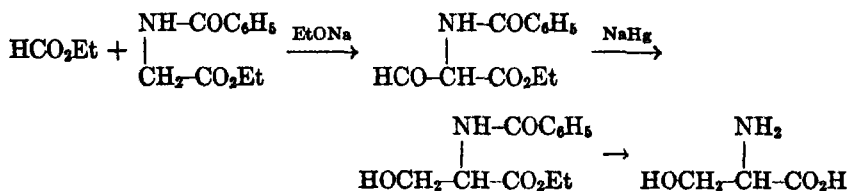
and from methyl acrylate [203] by the following series of reactions:

[197] Kapfhammer and Matthes, *Z. physiol. Chem.*, **223**, 43 (1934).
[198] Engeland, *Ber.*, **42**, 2962 (1909); *Z. physiol. Chem.*, **130**, 130 (1922).
[199] Schulze and Trier, *ibid.*, **67**, 59 (1910); Trier, *ibid.*, **67**, 324 (1910).
[200] Folch and Schneider, *J. Biol. Chem.*, **137**, 51 (1941).
[201] Dunn, Redemann, and Smith, *ibid.*, **104**, 511 (1934).
[202] Erlenmeyer and Stoop, *Ann.*, **337**, 236 (1904).
[203] Schilts and Carter, *J. Biol. Chem.*, **116**, 793 (1936); Carter and West, "Organic Syntheses," John Wiley & Sons, New York (1940), Vol. 20, p. 81.

$$\begin{array}{c} CH_2 \\ \| \\ CH \\ | \\ CO_2CH_3 \end{array} \xrightarrow[]{\text{Hg(OCOCH}_3)_2 + CH_3OH} \begin{array}{c} CH_2OCH_3 \\ | \\ CHHgOCOCH_3 \\ | \\ CO_2CH_3 \end{array} \xrightarrow[]{\text{KBr}} \begin{array}{c} CH_2OCH_3 \\ | \\ CHHgBr \\ | \\ CO_2CH_3 \end{array} \xrightarrow[]{\text{Br}_2}$$

$$\begin{array}{c} CH_2OCH_3 \\ | \\ CHBr \\ | \\ CO_2CH_3 \end{array} \xrightarrow[]{\text{NaOH; H}_2SO_4} \begin{array}{c} CH_2OCH_3 \\ | \\ CHBr \\ | \\ CO_2H \end{array} \xrightarrow[]{\text{NH}_3} \begin{array}{c} CH_2OCH_3 \\ | \\ CHNH_2 \\ | \\ CO_2H \end{array} \xrightarrow[]{\text{HBr; H}_2O; NH_3} \begin{array}{c} CH_2OH \\ | \\ CHNH_2 \\ | \\ CO_2H \end{array}$$
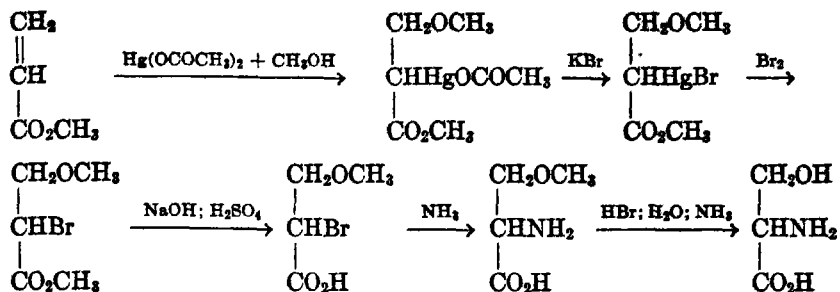
A variant of the second synthesis involves the preparation of ethyl α-bromo-β-ethoxypropionate by the action of sodium ethoxide on ethyl α,β-dibromopropionate.[204]

The resolution of racemic serine has been effected by fractional crystallization of alkaloid salts of its p-nitrobenzoyl derivative.[205] The configurational relationship of the natural $l(+)$ variety of serine to natural $l(+)$ alanine has been established [206] by direct conversion.

$$\begin{array}{c} NH_2 \\ | \\ HOCH_2-CH-CO_2H \end{array} \rightarrow \begin{array}{c} NH_2 \cdot HCl \\ | \\ HOCH_2-CH-CO_2CH_3 \end{array} \xrightarrow[]{\text{PCl}_5} \begin{array}{c} NH_2 \cdot HCl \\ | \\ ClCH_2-CH-CO_2CH_3 \end{array} \xrightarrow[]{\text{NaHg}}$$

$$\begin{array}{c} NH_2 \\ | \\ CH_3-CH-CO_2H \end{array}$$
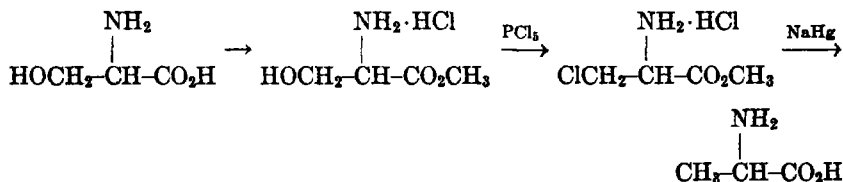
l-Serine has been produced [207] from l-asparagine by the following series of reactions:

$$\begin{array}{c} CO_2H \\ | \\ C_7H_7OCO-NH-C-H \\ | \\ CH_2 \\ | \\ CONH_2 \end{array} \xrightarrow[]{\text{NaOCl, HCl}} \begin{array}{c} CO_2H \\ | \\ C_7H_7OCO-N-C-H \\ \quad CO\diagdown \quad | \\ \quad\quad NH-CH_2 \end{array} \xrightarrow[]{\text{HCl}} \begin{array}{c} CO_2H \\ | \\ NH_2-C-H \\ | \\ CH_2NH_2 \end{array} \rightarrow$$

$$\begin{array}{c} CO_2H \\ | \\ C_7H_7OCO-NH-C-H \\ | \\ C_7H_7OCO-NH-CH_2 \end{array} \xrightarrow[]{\text{PCl}_5} \begin{array}{c} O-CO \\ CO\diagup \quad\diagdown \quad | \\ \quad NH-C-H \\ \quad\quad | \\ C_7H_7OCONH-CH_2 \end{array} \xrightarrow[]{\text{CH}_3OH} \begin{array}{c} CO_2CH_3 \\ | \\ NH_2-C-H \\ | \\ C_7H_7OCONH-CH_2 \end{array} \rightarrow$$

$$\begin{array}{c} CO_2CH_3 \\ | \\ C_6H_5CONH-C-H \\ | \\ C_7H_7OCO-NH-CH_2 \end{array} \xrightarrow[]{\text{H, Pt}} \begin{array}{c} CO_2CH_3 \\ | \\ C_6H_5CONH-C-H \\ | \\ CH_2NH_2 \end{array} \xrightarrow[]{\text{HNO}_2 \atop \text{HBr}} \begin{array}{c} CO_2H \\ | \\ NH_2-C-H \\ | \\ CH_2OH \end{array}$$

[204] Wood and du Vigneaud, *J. Biol. Chem.*, **134**, 413 (1940).
[205] Fischer and Jacobs, *Ber.*, **39**, 2942 (1906).
[206] Fischer and Raske, *Ber.*, **40**, 3717 (1907); **41**, 893 (1908).
[207] Schneider, *Ann.*, **529**, 1 (1937).

On boiling with sulfuric acid, serine slowly breaks down into ammonia and pyruvic acid:[208, 209]

$$HOCH_2-CH(NH_2)-CO_2H \rightarrow CH_2=C(NH_2)-CO_2H \rightarrow$$
$$CH_3-C(=NH)-CO_2H \rightarrow CH_3-CO-CO_2H + NH_3$$
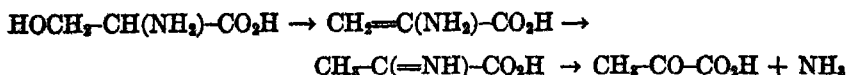
This change occurs more readily in alkaline solution [210] but is complicated by side reactions which lead to the production of oxalic acid, lactic acid, alanine and glycine. The formation of glycine has been attributed [211] to a reaction analogous to reversal of aldolization, which takes place more smoothly with phenylserine.[209]

$$C_6H_5-CHOH-CH(NH_2)-CO_2H \rightarrow C_6H_5CHO + CH_2(NH_2)-CO_2H$$
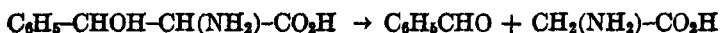
The conversion of serine to pyruvic acid has also been effected by reactions in which intermediate products have been isolated;[212] either by dehydration and subsequent acid hydrolysis of an azlactone;

$$\begin{array}{ccc} HOCH_2-CH-NH_2 & HOCH_2-CH-NH-CO-CH_3 & HOCH_2-CH-N=C-CH_3 \\ \quad\quad\mid & \quad\quad\mid & \quad\quad\mid\quad\quad\mid \\ \quad CO_2H & \quad CO_2H & \quad CO\text{----}O \end{array} \rightarrow$$

$$\begin{array}{cc} CH_2=C-N=C-CH_3 & CH_3CO \\ \quad\mid\quad\quad\mid & \quad\mid \\ \quad CO\text{----}O & \quad CO_2H \end{array} + NH_3 + CH_3CO_2H$$
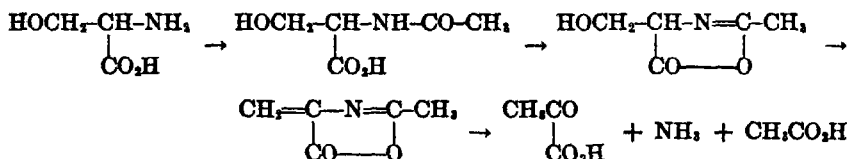
or by alkaline hydrolysis of the phenylhydantoin.

On treatment with nitrous acid serine yields, besides glyceric acid, small quantities of acetaldehyde.[209] It is quantitatively oxidized to formaldehyde by periodic acid [213] by way of glycollaldehyde.[200]

A phosphorus-containing amino acid having the composition of serine-phosphoric acid,

$$\begin{array}{l} CH(NH_2)-CO_2H \\ \mid \\ CH_2OPO(OH)_2 \end{array}$$
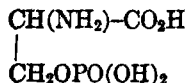
has been isolated [214] from the products of acid hydrolysis of vitellic acid, a hydrolysis product of the proteins of vitellin (from egg yolk) and casein.[215] The compound, which is slowly hydrolyzed in acid, rapidly in

[208] Erlenmeyer, *Ber.*, **35**, 3769 (1902).
[209] Bettzieche, *Z. physiol. Chem.*, **150**, 177 (1925).
[210] Daft and Coghill, *J. Biol. Chem.*, **90**, 341 (1931).
[211] Nicolet, *Science*, **74**, 250 (1931).
[212] Bergmann and Delis, *Ann.*, **458**, 76 (1927).
[213] Nicolet and Shinn, *J. Am. Chem. Soc.*, **61**, 1615 (1939).
[214] Levene and collaborators, *J. Biol. Chem.*, **98**, 109 (1932); **103**, 537 (1933); **105**, 547; **106**, 595 (1934).
[215] Lipmann, *Biochem. Z.*, **262**, 3, 9 (1933).

alkaline solution, has been synthesized by the action of phosphorus pentoxide in phosphoric acid upon *dl*-serine, followed by resolution of the brucine salts.

Threonine. A homolog of serine, α-amino-β-hydroxybutyric acid, is a constituent amino acid of many proteins, and is indispensable for growth in the diet of the rat.[216] On reduction with hydriodic acid and phosphorus it yields the $l(+)$-α-amino-*n*-butyric acid identical with that obtained from biological material. On oxidation successively with chloramine T and with bromine it is converted into $d(-)$-lactic acid.[217]

$$
\begin{array}{ccccccccc}
\text{CO}_2\text{H} & & \text{CO}_2\text{H} & & & \text{CHO} & & & \text{CO}_2\text{H} \\
| & & | & & & | & & & | \\
\text{NH}_2-\text{C}-\text{H} & \xleftarrow{\text{HI}+\text{P}} & \text{NH}_2-\text{C}-\text{H} & \xrightarrow{\text{Chl T}} & & \text{H}-\text{C}-\text{OH} & \xrightarrow{\text{Br}_2} & & \text{H}-\text{C}-\text{OH} \\
| & & | & & & | & & & | \\
\text{CH}_2 & & \text{H}-\text{C}-\text{OH} & & & \text{CH}_3 & & & \text{CH}_3 \\
| & & | & & & & & & \\
\text{CH}_3 & & \text{CH}_3 & & & & & &
\end{array}
$$
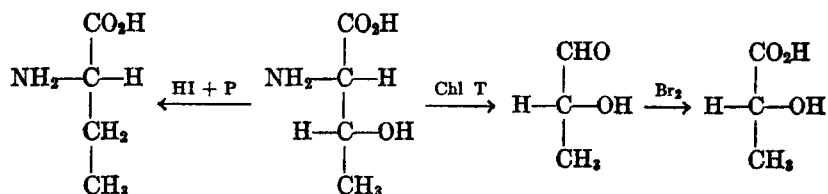
The constitution thereby established is supported by the behavior with nitrous acid, which with α-amino acids brings about the replacement of amino by hydroxyl groups without Walden inversion:[218] the threo-dihydroxybutyric acid corresponding to $d(-)$-threose is produced. It is proposed that the natural α-amino-β-hydroxybutyric acid, although its configuration at the α carbon atom is that of other natural amino acids, be termed $d(-)$-threonine so as to show the stereochemical relationship of the grouping at the β position to that of $d(-)$-threose.

Natural threonine has been synthesized[219] from crotonic acid by a process similar to that employed for serine (p. 1121).

$$
\begin{array}{c}
\text{CH}_3\text{-CH}=\text{CH-CO}_2\text{H} \rightarrow \text{CH}_3\text{-CH}\underset{\underset{\text{OCH}_3}{|}}{\makebox[2em]{}}\text{CH-CO}_2\text{H} \rightarrow \\
\phantom{\text{CH}_3\text{-CH}=\text{CH-CO}_2\text{H} \rightarrow \text{CH}_3\text{-CH}}\underset{\underset{\text{HgBr}}{|}}{\makebox[2em]{}}
\end{array}
$$

$$
\text{CH}_3\text{-CH}\underset{\underset{\text{OCH}_3}{|}}{\makebox[2em]{}}\underset{\underset{\text{Br}}{|}}{}\text{CH-CO}_2\text{H} \rightarrow \text{CH}_3\text{-CH}\underset{\underset{\text{OCH}_3}{|}}{\makebox[2em]{}}\underset{\underset{\text{NH}_2}{|}}{}\text{CH-CO}_2\text{H}
$$
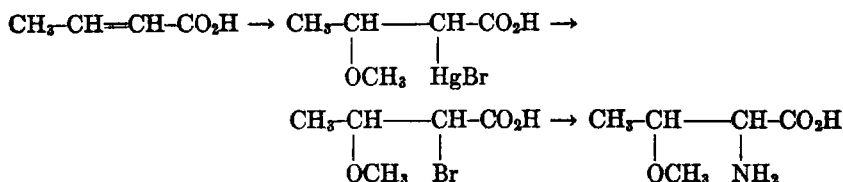
The resulting *dl*-O-methylthreonine and *dl*-O-methylallothreonine were separated after acylation, and each racemic product resolved by means of brucine. The four stereoisomers so secured were demethylated to the corresponding threonines by boiling with hydrobromic acid.

In a second synthesis,[220] acetaldehyde and hippuric acid were condensed in the presence of acetic anhydride, and the resulting azlactone

[216] McCoy, Meyer, and Rose, *J. Biol. Chem.*, **112**, 283 (1935).

[217] Meyer and Rose, *ibid.*, **115**, 721 (1936).

[218] Levene, *Chem. Rev.*, **2**, 179 (1926).

[219] West and Carter, *J. Biol. Chem.*, **119**, 103, 109 (1937).

[220] Carter, Handler, and Melville, *ibid.*, **129**, 359 (1939).

was converted into the methyl ester of N-benzoyl-O-methylthreonine by the action of sodium methoxide:

$$CH_3CHO + \underset{\underset{NHCOC_6H_5}{|}}{CH_2-CO_2H} \xrightarrow{Ac_2O} \underset{\underset{N=C}{|}}{\overset{CH_3-CH=C-CO}{\underset{C_6H_5}{\diagdown O}}} \xrightarrow{CH_3ONa}$$

$$\underset{\underset{CH_3}{|}}{\underset{H-C-OCH_3}{|}}\overset{\overset{CO_2CH_3}{|}}{C_6H_5CONH-C-H} + \underset{\underset{CH_3}{|}}{\underset{CH_3O-C-H}{|}}\overset{\overset{CO_2CH_3}{|}}{H-C-NHCOC_6H_5}$$

In this synthesis, very little of the allothreonine derivative (from which no natural threonine can be secured on hydrolysis and demethylation) is formed.

An azlactone identical with the above is formed on treating the benzoyl derivative of either threonine, allothreonine, or their methyl ethers with acetic anhydride or with pyridine and benzoyl chloride in the cold; on the other hand, when N-benzoyl-O-methylallothreonine is treated in acetic acid with acetic anhydride, a second, isomeric, azlactone is simultaneously formed. These isomers, which are of the *cis* and *trans* varieties, yield on hydrolysis the corresponding geometrically isomeric benzoyl-α-aminocrotonic acids.

Threonine is oxidized by periodic acid, with formation of acetaldehyde.[213] On methylation, threonine and allothreonine yield betaines which on treatment with alkali undergo retrograde aldolization, yielding acetaldehyde and the betaine of glycine.[221]

$$\underset{\underset{CH_3-CHOH-CH-CO_2^-}{|}}{\overset{\overset{+}{N(CH_3)_3}}{}} \rightarrow CH_3CHO + \underset{}{\overset{\overset{+}{N(CH_3)_3}}{}}CH_2-CO_2^-$$

Hydroxyglutamic Acid. The dicarboxylic acid fractions of hydrolyzed casein and zein have been shown by Dakin [222] to contain, besides aspartic and glutamic acid, a (+)-hydroxyglutamic acid, which is weakly dextrorotatory in water and more strongly so in acid. It appears to be a constituent of a phosphopeptone from casein.[223] Owing to the technical difficulties involved in its isolation [224] the natural product has received little attention.

[221] Carter and Melville, *ibid.*, **133**, 109 (1940).

[222] Dakin, *Biochem. J.*, **12**, 290 (1918); *Z. physiol. Chem.*, **130**, 159 (1923).

[223] Rimington, *Biochem. J.*, **21**, 1179, 1187 (1927).

[224] Gulland and Morris, *J. Chem. Soc.*, 1644 (1934).

Two syntheses of inactive β-hydroxyglutamic acid have been devised. The first of these [225] starts with glutamic acid.

$$
\begin{array}{c}
CO_2H \\
| \\
CHNH_2 \\
| \\
CH_2 \\
| \\
CH_2 \\
| \\
CO_2H
\end{array}
\quad \xrightarrow{\text{KOCN, HCl}} \quad
\begin{array}{c}
CO-NH \\
| \quad\quad \searrow CO \\
CH-NH \nearrow \\
| \\
CH_2 \\
| \\
CH_2 \\
| \\
CO_2H
\end{array}
\quad \xrightarrow{\text{Br}_2} \quad
\begin{array}{c}
CO-NH \\
| \quad\quad \searrow CO \\
CH-NH \nearrow \\
| \\
CHBr \\
| \\
CH_2 \\
| \\
CO_2H
\end{array}
\quad \xrightarrow{\text{H}_2O}
$$

$$
\begin{array}{c}
CO-NH \\
| \quad\quad \searrow CO \\
CH-NH \nearrow \\
| \\
CH \\
\| \\
CH \\
| \\
CO_2H
\end{array}
\quad \xrightarrow{\text{Ba(OH)}_2} \quad
\begin{array}{c}
CO_2H \\
| \\
CH-NH_2 \\
| \\
CHOH \\
| \\
CH_2 \\
| \\
CO_2H
\end{array}
$$

The second, and more convenient, synthesis [226] involves the catalytic reduction of ethyl isonitrosoacetonedicarboxylate:

$$
\begin{array}{c}
CO_2Et \\
| \\
CH_2 \\
| \\
CO \\
| \\
CH_2 \\
| \\
CO_2Et
\end{array}
\;\rightarrow\;
\begin{array}{c}
CO_2Et \\
| \\
C=NOH \\
| \\
CO \\
| \\
CH_2 \\
| \\
CO_2Et
\end{array}
\;\rightarrow\;
\begin{array}{c}
CO_2Et \\
| \\
CHNH_2 \\
| \\
CHOH \\
| \\
CH_2 \\
| \\
CO_2Et
\end{array}
\;\rightarrow\;
\begin{array}{c}
CO_2H \\
| \\
CHNH_2 \\
| \\
CHOH \\
| \\
CH_2 \\
| \\
CO_2H
\end{array}
$$

The synthetic product consists of a mixture of two racemic diastereoisomers, the separation of which has not been accomplished.

Hydroxyproline. $l(-)$-γ-Hydroxyproline, which has been stated [227] to occur in combination in proteins of animal, but rarely in those of vegetable origin, resembles proline in its solubility in alcohols. Its copper salt, soluble in methyl alcohol, differs from that of proline by being insoluble in absolute ethyl alcohol.[228] Like proline, hydroxyproline is precipitable by Reinecke salt; on the other hand, the rhodanilate is soluble.

[225] Dakin, *Biochem. J.*, **13**, 398 (1919).
[226] Harington and Randall, *ibid.*, **25**, 1917 (1931).
[227] Spörer and Kapfhammer, *Z. physiol. Chem.*, **187**, 84 (1930).
[228] Klabunde, *J. Biol. Chem.*, **90**, 293 (1931).

Its relation to proline was ascertained by Fischer, at the time of its discovery,[229] by its conversion into proline on heating with phosphorus and hydriodic acid. The position of the hydroxyl group was subsequently established by synthesis.[230]

$$\underset{\underset{\text{ClCH}_2\text{-CH-CH}_2}{}}{\overset{\text{O}}{\triangle}} + \text{CHNa(CO}_2\text{Et)}_2 \rightarrow \underset{\underset{\text{O}\text{------CO}}{}}{\text{ClCH}_2\text{-CH-CH}_2\text{-CH-CO}_2\text{Et}} \overset{\text{Cl}_2}{\longrightarrow}$$

$$\underset{\underset{\text{O}\text{------CO}}{}}{\text{ClCH}_2\text{-CH-CH}_2\text{-CCl-CO}_2\text{Et}} \rightarrow \underset{\underset{\text{O}\text{------CO}}{}}{\text{ClCH}_2\text{-CH-CH}_2\text{-CHCl}} \overset{\text{NH}_3}{\longrightarrow}$$

$$\underset{\text{HOCH-CH}_2\text{-CH-CO}_2\text{H}}{\overset{\text{CH}_2\text{------NH}}{|\qquad\qquad|}}$$
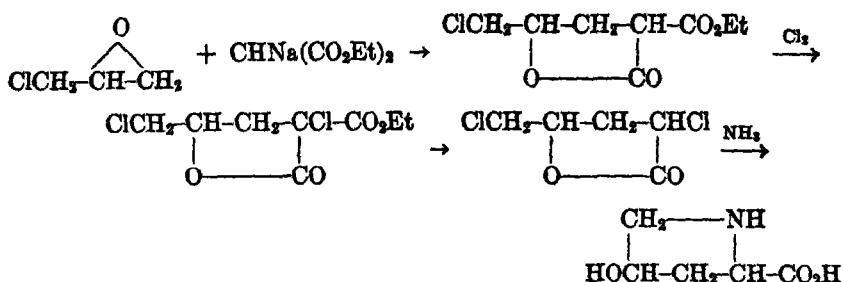
The resulting inactive mixture of diastereoisomers was separated, by crystallization of the copper salts, into two racemic compounds. One of these was converted into the phenylhydantoic acid; the quinine salt of this on fractional crystallization yielded a product identical with the corresponding derivative of the natural amino acid.

$$\underset{\text{HOCH-CH}_2\text{-CH-CO}_2\text{H}}{\overset{\text{CH}_2\text{------N-CONHC}_6\text{H}_5}{|\qquad\qquad|}} + \text{NH}_3 \rightarrow \underset{\text{HOCH-CH}_2\text{-CH-CO}_2\text{H}}{\overset{\text{CH}_2\text{------NH}}{|\qquad\qquad|}} + \text{NH}_2\text{CONHC}_6\text{H}_5$$
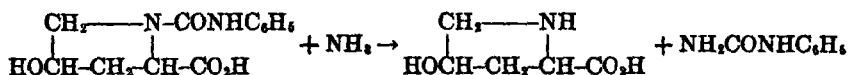
In a similar synthesis, subsequently performed,[231] the terminal chlorine atom was exchanged for an amino group prior to bromination at the α position.

On treatment with sodium hypochlorite, hydroxyproline yields a volatile product which gives color reactions with dimethylaminobenzaldehyde and with isatin.[232] This is pyrrole.[233]

$$\underset{\text{HOCH------CH}_2}{\overset{\text{CH}_2\text{-NH-CH-CO}_2\text{H}}{|\qquad\qquad|}} \rightarrow \underset{\text{CH------CH}}{\overset{\text{CH-NH-CH}}{||\qquad\qquad||}} + \text{CO}_2$$
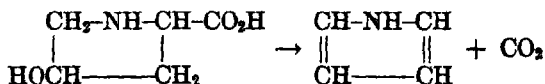
Tyrosine. $l(-)$-Tyrosine is very widely distributed as a protein component; however, it is not present in gelatin. Its presence, in combination, is mainly responsible for the yellow color developed by proteins with nitric acid followed by ammonia or alkali (xanthoproteic reaction), and the pink color produced by Millon's reagent (a solution of mercurous nitrate in nitric acid). The Millon test is characteristic for phenols con-

[229] Fischer, *Ber.*, **35**, 2660 (1902).

[230] Leuchs and collaborators, *Ber.*, **38**, 1937 (1905); **41**, 1726 (1908); **45**, 1960 (1912); **46**, 986 (1913).

[231] Traube, Johow, and Tepohl, *Ber.*, **56**, 1861 (1923).

[232] Lang, *Z. physiol. Chem.*, **219**, 148 (1933).

[233] Waldschmidt-Leitz and Akabori, *ibid.*, **224**, 187 (1934).

taining a free *ortho* position. Tyrosine also yields a red color on treatment in alkaline solution with diazobenzenesulfonic acid [234] and green or red colors with sulfuric acid solutions of formaldehyde or acetaldehyde respectively.[235, 236] Quantitative color tests, applicable to the estimation of small quantities of tyrosine, depend upon the formation of a blue color with alkaline phosphomolybdate solution [237] and upon a standardized application of Millon's test.[238]

The biochemical conversion of phenylalanine into tyrosine in normal rats has been unequivocally demonstrated [239] by the isolation of tyrosine containing deuterium in the aromatic nucleus from the bodies of animals, the normal diet of which had been supplemented by deuterophenylalanine.

The synthesis of *dl*-tyrosine has been accomplished by the hippuric acid method [240] and by the introduction of hydroxyl into phenylalanine [241] by successive nitration, reduction, and diazotization. This second method is of interest as exemplifying the possibility of diazotizing an amino group attached to an aromatic nucleus without affecting another present in a side chain.

When heated under reduced pressure, or better in a mixture of diphenylmethane and diphenylamine at 260–265° under atmospheric pressure,[40] tyrosine loses carbon dioxide with formation of tyramine (β-*p*-hydroxyphenylethylamine).

The configurational relationship of natural *l*(−)-tyrosine to other amino acids has been confirmed by oxidation of the N-benzoyl derivative, which yields the benzoyl derivative of natural *l*-aspartic acid.[242]

$$
\begin{array}{ccc}
C_6H_4OH & & CO_2H \\
| & & | \\
CH_2 & & CH_2 \\
| & \rightarrow & | \\
H-C-NHCOC_6H_5 & & H-C-NHCOC_6H_5 \\
| & & | \\
CO_2H & & CO_2H
\end{array}
$$
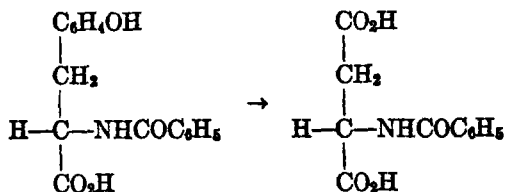
When tyrosine is subjected to the action of air in presence of the enzyme tyrosinase, which is found in many animal and vegetable

[234] Pauly, *ibid.*, **42**, 508 (1904).
[235] Denigès, *Compt. rend.*, **130**, 583 (1900); *Bull. soc. chim.*, [4] **3**, 786 (1908).
[236] Mörner, *Z. physiol. Chem.*, **37**, 86 (1902).
[237] Folin and Marenzi, *J. Biol. Chem.*, **83**, 89 (1929).
[238] Folin and Ciocalteu, *ibid.*, **73**, 627 (1927).
[239] Moss and Schoenheimer, *ibid.*, **135**, 415 (1940).
[240] Erlenmeyer and Halsey, *Ann.*, **307**, 138 (1899).
[241] Erlenmeyer and Lipp, *Ann.*, **219**, 161 (1883).
[242] Goldschmidt and Freyss, *Ber.*, **66**, 784 (1933).

tissues (e.g., mealworms or potatoes), oxidation occurs, a dark amorphous, weakly acidic pigment called melanin being formed as the final product. Of the series of reactions which takes place, the first is the introduction of a hydroxyl group in the position *ortho* to that in the tyrosine.[243] The product, $l(-)$-3,4-dihydroxyphenylalanine, has been isolated from extracts of various vegetable and animal tissues, but has never been obtained from protein hydrolysates. The racemic variety, synthesized by standard processes, has been resolved into its optically active components by crystallizing the brucine salts of the acetyl derivative prepared by hydrogenating the condensation product of protocatechualdehyde and acetylglycine.[244]

Being an *o*-dihydroxylic phenol, dihydroxyphenylalanine is readily autoxidizable in alkaline solution, or in neutral solution in the presence of a specific oxidase, with formation of melanin. Apparently the only reaction specifically induced by tyrosinase is the introduction of the hydroxyl group; this reaction, however, also takes place with other phenols. Raper [243, 245] has shown that the production of melanin involves the intermediate formation of derivatives of indole.
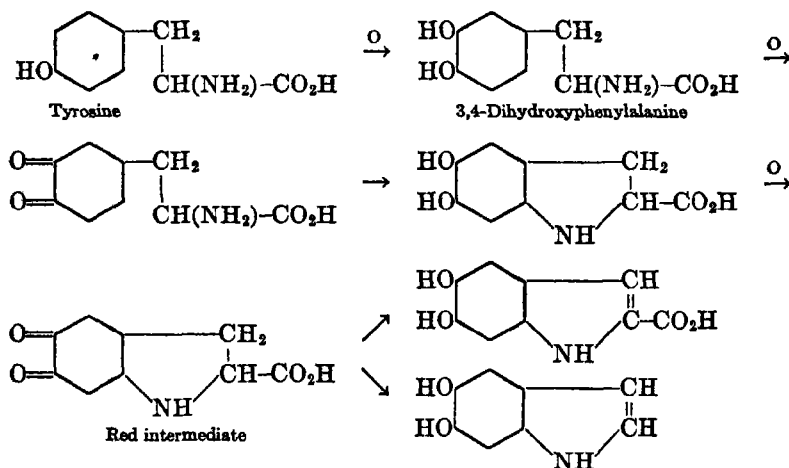
The first two steps in the above system require the presence of enzymes, the subsequent reactions do not.[246] The chemical nature of melanin is still obscure; it appears to represent a further stage in the oxidation of 5,6-dihydroxyindole. Melanin or similar products are formed, though more slowly, by the action of tyrosinase upon substances

[243] Raper, *Biochem. J.*, **20**, 735 (1926).
[244] Harington and Randall, *ibid.*, **25**, 1028 (1931).
[245] Raper and collaborators, *ibid.*, **21**, 89, 1370 (1927); **29**, 76 (1935).
[246] Evans and Raper, *ibid.*, **31**, 2162 (1937).

such as tyramine and N-methyltyrosine, closely allied to tyrosine. Tyrosol (β-p-hydroxyphenylethanol) yields a red product, presumably an o-quinone.

Like other phenols, tyrosine responds readily to substitution, yielding with chlorine or bromine the corresponding dihalogenated tyrosine. Dibromotyrosine gives no color with Millon's reagent, diazo compounds, or formaldehyde in sulfuric acid, but responds to the phosphomolybdate, ninhydrin (p. 1099), and xanthoproteic tests. Its isolation from the products of alkaline hydrolysis of a Norwegian coral has been reported.[247]

3,5-Diiodotyrosine (iodogorgoic acid), which can be synthesized by the action of iodine upon tyrosine in weakly alkaline solution,[248] is also present, in combined form, in proteins which have been artificially iodinated [249] and in certain natural proteins. It was first isolated [250] from the axial skeleton of a coral (*Gorgonia cavolini*), later from sponge [251, 252] and from thyroglobulin, the iodine-containing protein of the thyroid gland.[253, 254]

Diiodotyrosine breaks down, with loss of iodine, in boiling acid solution, but is stable to alkali; hydrolysis of iodine-containing proteins by barium hydroxide leads to racemization, and the product, though it closely resembles that obtained by iodination of *l*-tyrosine, actually is identical with the racemic variety.[255] The natural isomer, however, has been obtained [256] by enzymatic hydrolysis of thyroglobulin.

Thyroxine. This iodine-containing amino acid, which exerts the stimulating effect on metabolism characteristic of thyroglobulin, was first isolated by Kendall [257] from an alkali hydrolysate of thyroid substance. Its composition and constitution were determined by Harington,[258] who first established the identity of thyronine (the iodine-free product obtained by catalytic reduction) with the synthetic p-hydroxyphenyl ether of tyrosine,

$$HO-\underset{}{\bigcirc}-O-\underset{}{\bigcirc}-CH_2-\underset{CO_2H}{\overset{}{CHNH_2}}$$
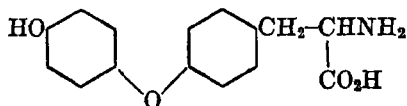
[247] Mörner, *Z. physiol. Chem.*, **88**, 140 (1913).
[248] Wheeler and Jamieson, *Am. Chem. J.*, **33**, 365 (1905).
[249] Oswald, *Z. physiol. Chem.*, **70**, 310 (1910); **71**, 200; **74**, 290 (1911).
[250] Drechsel, *Z. Biol.*, **33**, 85 (1896).
[251] Wheeler and Mendel, *J. Biol. Chem.*, **7**, 1 (1909).
[252] Oswald, *Z. physiol. Chem.*, **75**, 353 (1911).
[253] Harington and Randall, *Biochem. J.*, **23**, 373 (1929).
[254] Foster, *J. Biol. Chem.*, **83**, 345 (1929).
[255] Henze, *Z. physiol. Chem.*, **51**, 64 (1907).
[256] Harington and Randall, *Biochem. J.*, **25**, 1032 (1931).
[257] Kendall, *J. Am. Med. Assoc.*, **64**, 2042 (1915); *J. Biol. Chem.*, **39**, 125 (1919).
[258] Harington, *Biochem. J.*, **20**, 293, 300 (1926).

and subsequently, with Barger,[259] synthesized thyroxine itself by condensing the aldehyde obtained by the following steps:

$$CH_3O \langle \rangle OH + I \langle \rangle^{I}_{I} NO_2 \rightarrow CH_3O \langle \rangle O \langle \rangle^{I}_{I} NO_2 \rightarrow$$

$$CH_3O \langle \rangle O \langle \rangle^{I}_{I} NH_2 \rightarrow CH_3O \langle \rangle O \langle \rangle^{I}_{I} CN \rightarrow CH_3O \langle \rangle O \langle \rangle^{I}_{I} CHO$$
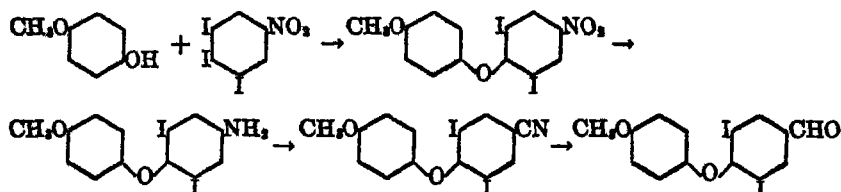
with hippuric acid, reducing and demethylating the product by heating with hydriodic acid and phosphorus, and finally iodinating the phenolic group.

The racemic thyroxine so prepared was identical with that obtained from thyroid, for the instability of thyroxine toward boiling acids rendered necessary the use of barium hydroxide as hydrolysant, with consequent racemization. The optically active isomers were prepared by resolution of the formyl derivative of 3,5-diiodothyronine (the product of the penultimate step in the above synthesis) followed by hydrolysis and iodination. Of these, the levorotatory variety has the same configuration as natural $l(-)$-tyrosine; this relationship was established [260] by comparison of the corresponding thyronine with a sample synthesized from N-benzoyl-l-tyrosine ester and 3,4,5-triiodonitrobenzene, with subsequent removal of the iodine atoms by hydrogen and palladium, and replacement of the amino (originally nitro) group by hydroxyl.

Enzymatic hydrolysis of thyroglobulin leads to l-thyroxine [261] which is twice as active physiologically as the racemic product.[262] The same relation holds between the respective potencies of thyroxine in naturally combined form (thyroid protein) and dl-thyroxine, from which it appears probable that the physiological activity of the latter is due almost entirely to the *levo* component. The observation of some activity in d-thyroxine may conceivably be attributable to its biological conversion to l-thyroxine through the corresponding α-keto acid, which has been shown [263] to exert, though to a lower degree, the specific physiological action of thyroxine.

Cysteine and Cystine. These sulfur-containing amino acids, structurally closely related to serine, occur almost universally in proteins; in gelatin, however, they are present only in traces. Although they are

[259] Harington and Barger, *ibid.*, **21**, 169 (1927).
[260] Harington and collaborators, *ibid.*, **22**, 1429 (1928); **28**, 68 (1934).
[261] Harington and Salter, *ibid.*, **24**, 456 (1930).
[262] Foster, Palmer, and Leland, *J. Biol. Chem.*, **115**, 467 (1936).
[263] Canzanelli, Guild, and Harington, *Biochem. J.*, **29**, 1617 (1935).

readily interconvertible, it has been found possible,[5] by the application of principles discussed below, to demonstrate the extent to which the one or the other is present in a given protein or its hydrolysate. $l(-)$-Cystine is found as such in the urine of persons subject to the obscure metabolic disturbance known as cystinuria, and it forms the major constituent of the urinary calculi often associated with this condition.

$l(+)$-Cysteine has been synthesized by the action of phosphorus pentasulfide on benzoylserine ester, followed by hydrolysis,[202] and from serine ester hydrochloride by successive treatment with phosphorus pentachloride and barium hydrosulfide.[264] The cysteine so produced from natural serine yields on gentle oxidation the natural, levorotatory l-cystine.

A convenient synthesis of the racemic variety of cysteine has been devised by Wood and du Vigneaud.[265] Chloromethyl benzyl sulfide, from benzyl mercaptan, formaldehyde, and hydrogen chloride, is condensed with phthalimidomalonic ester (p. 1105); the product on hydrolysis yields S-benzyl-dl-cysteine,

$$C_6H_5CH_2SH + CH_2O + HCl \rightarrow C_6H_5CH_2SCH_2Cl \rightarrow$$

$$C_6H_5CH_2SCH_2C(CO_2C_2H_5)_2N\diagup\!\!\!\!\overset{CO}{\underset{CO}{\diagdown}}\!\!\!\!\diagdown C_6H_4 \rightarrow C_6H_5CH_2SCH_2CH(NH_2)CO_2H$$
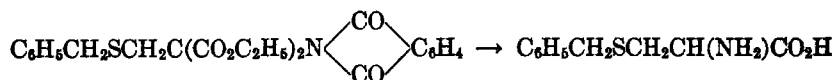
from which the benzyl group is removed by sodium in liquid ammonia:

$$
\begin{array}{c}
CH_2SCH_2C_6H_5 \\
| \\
2CHNH_2 \\
| \\
CO_2H
\end{array}
\quad + 2Na \rightarrow
\begin{array}{c}
CH_2SNa \\
| \\
2CHNH_2 \\
| \\
CO_2H
\end{array}
+ C_6H_5CH_2CH_2C_6H_5
$$
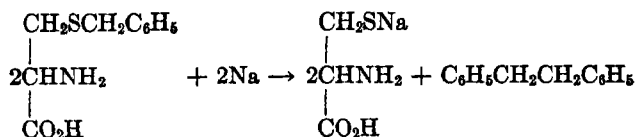
The interconversion of cysteine and cystine

$$
2\begin{array}{c}
CH_2SH \\
| \\
H-C-NH_2 \\
| \\
CO_2H
\end{array}
\;\underset{H}{\overset{O}{\rightleftarrows}}\;
\begin{array}{cc}
CH_2-S\!-\!\!-\!\!-\!S-CH_2 \\
| \qquad\qquad | \\
H-C-NH_2 \quad H-C-NH_2 \\
| \qquad\qquad | \\
CO_2H \qquad\quad CO_2H
\end{array}
$$
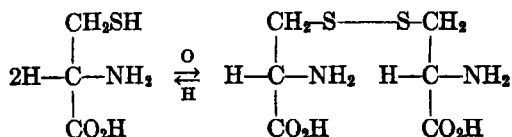
is a thermodynamically reversible process, the oxidation-reduction potential of which is apparently characteristic of the general system

$$2H + RS\text{–}SR \rightleftarrows 2RSH$$

and independent of the nature of the group R.[266]

[264] Fischer and Raske, *Ber.*, **41**, 893 (1908).
[265] Wood and du Vigneaud, *J. Biol. Chem.*, **131**, 267 (1939).
[266] Fruton and Clarke, *ibid.*, **106**, 667 (1934).

Reduction of cystine to cysteine is readily effected, in acid solutions, by means of tin or zinc; in neutral or alkaline solutions an excess of another sulfhydryl compound, such as thioglycollic acid, may be employed for the reduction of the disulfide linkage in proteins.[267]

$$RSSR + 2R'SH \rightleftarrows 2RSH + R'SSR'$$

For preparative purposes, the action of metallic sodium in liquid ammonia is often convenient.[268]

Oxidation of cysteine to cystine may be effected by oxygen in faintly alkaline solution, a reaction catalyzed by traces of salts of iron and other metals which form autoxidizable complexes with cysteine.[269] It can also be carried out in acid solution by means of iodine; this reaction forms the basis of a method for the quantitative estimation of cysteine.[270]

When *dl*-cysteine is oxidized to the disulfide, or when *l*-cystine is racemized by prolonged boiling with hydrochloric acid,[271] the resulting optically inactive cystine consists of a mixture of the racemic and *meso* varieties.[272] Only the former can be resolved into active components:

dl-Cystine   *meso*-Cystine
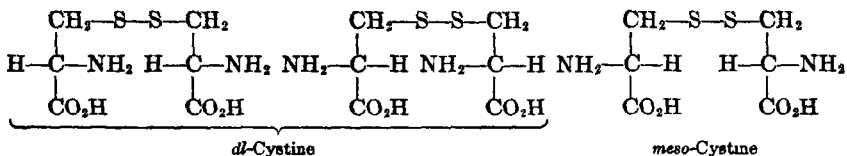
With bromine water, the sulfur atoms in cysteine (and cystine) are rapidly oxidized to sulfonic acid groups, with production of cysteic acid.

$$HO_3S\text{-}CH_2\text{-}CH(NH_2)\text{-}CO_2H$$

The same reaction takes place, relatively slowly, with iodine.[273]

Intermediate oxidation products have been isolated. By the action of perbenzoic acid in acetonitrile, cystine perchlorate is converted into a disulfoxide, which is reduced to cystine by hydriodic acid and oxidized to cysteic acid by excess of iodine. In acid solution, spontaneous dismu-

[267] du Vigneaud and collaborators, *ibid.*, **94**, 233 (1931); Goddard and Michaelis, *ibid.*, **112**, 361 (1935).

[268] du Vigneaud, Audrieth, and Loring, *J. Am. Chem. Soc.*, **52**, 4500 (1930).

[269] Michaelis and Schubert, *ibid.*, **52**, 4418 (1930); **53**, 3851 (1931); Schubert, *ibid.*, **54**, 4077 (1932).

[270] Okuda, *J. Biol. Chem. (Japan)*, **5**, 207 (1925); *Proc. Imp. Acad. (Tokyo)*, **5**, 246 (1929); Lavine, *J. Biol. Chem.*, **109**, 141 (1935).

[271] Hoffmann and Gortner, *J. Am. Chem. Soc.*, **44**, 341 (1922).

[272] du Vigneaud and collaborators, *J. Biol. Chem.*, **94**, 243 (1931); **98**, 577 (1932); **102**, 287 (1933); **107**, 267 (1934).

[273] Friedmann, *Beitr. chem. Physiol. Path.*, **3**, 27 (1903); Yamazaki, *J. Biochem. (Japan)*, **12**, 207 (1930).

tation of the disulfoxide occurs [274] with formation of cystine and a sulfinic acid,

$$HO_2S-CH_2-CH(NH_2)-CO_2H$$

which is also formed by the action of hydrogen peroxide upon the complex potassium cobalto biscysteinate.[275] The dismutation of the disulfoxide, which is paralleled by the actions of p-thiocresol and sodium cyanide,

$$2RSO-SOR + 2C_7H_7SH \rightarrow RS-SR + 2RSO_2H + C_7H_7S-SC_7H_7$$

$$RSO-SOR + NaCN \rightarrow RSCN + RSO_2H$$
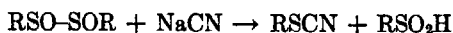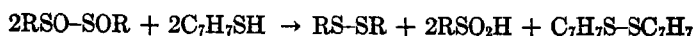
possibly involves a highly reactive and unstable sulfenic acid.

$$RSO-SOR + H_2O \rightarrow RSO_2H + RSOH$$

$$2RSOH \rightarrow RSH + RSO_2H$$

$$RSOH + RSH \rightarrow RS-SR$$
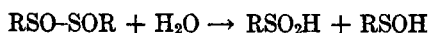
The reaction between cysteine and the hypothetical sulfenic acid appears to form one aspect of an equilibrium reaction undergone by cystine and other disulfides, especially in alkaline solution.[276]

$$RSSR + H_2O \rightleftarrows RSH + RSOH$$
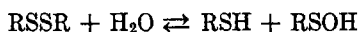
This equilibrium [277] serves to explain the behavior of cystine toward cyanide,[278]

$$RSSR + NaCN \rightarrow RSNa + RSCN$$

and sulfite.[279]

$$RSSR + Na_2SO_3 \rightarrow RSNa + RSSO_3Na$$
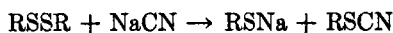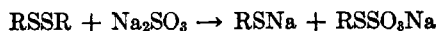
Like other thiol compounds, cysteine forms water-insoluble derivatives with silver, mercury, and cuprous copper. Such precipitates are also produced from cystine; with silver and mercuric ions, reduction of the disulfide linkage takes place at the expense of a portion (about one-sixth) which is not precipitated but appears as cysteic acid; [280] with excess of cuprous chloride, on the other hand, a quantitative yield is obtainable as the disulfide linkage is reduced by the cuprous ion.[281]

[274] Toennies and Lavine, *J. Biol. Chem.* **113**, 571, 583 (1936).

[275] Schubert, *J. Am. Chem. Soc.*, **55**, 3336 (1933).

[276] Schöberl and collaborators, *Ann.*, **507**, 111 (1933); *Ber.*, **67**, 1545 (1934); *Naturwissenschaften*, **24**, 391 (1935).

[277] Shinohara and Kilpatrick, *J. Biol. Chem.*, **105**, 241 (1934).

[278] Mauthner, *Z. physiol. Chem.*, **78**, 28 (1912); Pulewka and Winzer, *Arch. exptl. Path. Pharmakol.*, **138**, 154 (1928).

[279] Clarke, *J. Biol. Chem.*, **97**, 235 (1932).

[280] Vickery and Leavenworth, *ibid.*, **86**, 129 (1930); Simonsen, *ibid.*, **94**, 323 (1931).

[281] Rossouw and Wilken-Jorden, *Biochem. J.*, **29**, 219 (1935); Lucas and Beveridge, *ibid.*, **34**, 1356 (1940).

In colorimetric methods for the analytical estimation of cystine the first step consists in the rupture of the disulfide linkage to produce cysteine. The procedures elaborated by Folin,[287] and the modifications of these,[282] depend upon the blue color produced by the action of cysteine (and other sulfhydryl compounds) upon phosphotungstic acid. Lugg [283] has shown that the intensity of this color from pure cysteine alone is the same as that from a corresponding quantity of cystine in presence of sodium sulfite in excess; cysteine with sulfite yields twice the intensity. From this it is clear that, by the oxidizing action of the phosphotungstic reagent in the presence of sulfite, all the cystine (or cysteine) is ultimately converted into the non-reducing thiosulfonic derivative:

$$RSSR + RSSR \xrightarrow{Na_2SO_3} 2RSH + 2RSSO_3Na$$
$$RSSR \xleftarrow{PW \ acid} 2RSH$$
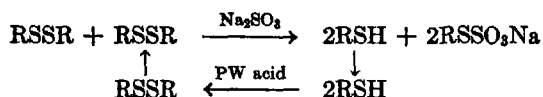
The development of sulfhydryl compounds of a characteristic purple color with nitroprusside has been applied to the estimation of cystine.[284] In this case the disulfide linkage is opened by double decomposition with cyanide.

More specific is the process of Sullivan.[285] In this, cystine is treated successively with cyanide and 1,2-naphthoquinone-4-sulfonic acid; the color developed by cysteine is not, in contrast to that from other amino acids, discharged on addition of sodium hydrosulfite. As in the Folin process, cysteine yields twice the color intensity furnished by cystine.[286] Slight variations in technique affect the color intensity. Under certain conditions exposure of the final solution to air leads to increase in color;[287] it seems possible that the reaction may involve an autoxidizable leuco derivative. A color reaction, possibly analogous to the preceding, is given by cysteine with o-benzoquinone.[288]

Derivatives of cysteine in which the sulfhydryl hydrogen atom is replaced by alkyl groups are of course devoid of actual or potential reducing properties and do not respond to the specific color tests. They may conveniently be synthesized by the action of alkyl halides upon the sodium derivative of cysteine in aqueous [289] or liquid ammonia [290] solu-

[282] Tompsett, *ibid.*, **25**, 2014 (1931); Shinohara, *J. Biol. Chem.*, **109**, 665 (1935).

[283] Lugg, *Biochem. J.*, **26**, 2144 (1932).

[284] Brand, Harris, and Biloon, *J. Biol. Chem.*, **86**, 315 (1930).

[285] Sullivan, *U. S. Pub. Health Repts.*, **41**, 1030 (1926); **44**, 1421 (1929); Sullivan and Hess, *ibid.*, **44**, 1599 (1929).

[286] Lugg, *Biochem. J.*, **27**, 668 (1933).

[287] Bushill, Lampitt, and Baker, *ibid.*, **28**, 1293 (1934).

[288] Dyer and Baudisch, *J. Biol. Chem.*, **96**, 483 (1932).

[289] Clarke and Inouye, *ibid.*, **94**, 541 (1931).

[290] du Vigneaud, Loring, and Craft, *ibid.*, **105**, 481 (1934).

tion. An interesting member of this series is $l(-)$-djenkolic acid, present in the Djenkol bean,[291] the synthesis of which from l-cysteine has been accomplished by the liquid ammonia technique.[292]

$$2\text{H-}\underset{\underset{\text{CO}_2\text{H}}{|}}{\overset{\overset{\text{CH}_2\text{SNa}}{|}}{\text{C-NH}_2}} + \text{CH}_2\text{Cl}_2 \rightarrow \text{H-}\underset{\underset{\text{CO}_2\text{H}}{|}}{\overset{\overset{\text{CH}_2\text{S-CH}_2\text{-SCH}_2}{|}}{\text{C-NH}_2}} \quad \text{H-}\underset{\underset{\text{CO}_2\text{H}}{|}}{\overset{\overset{|}{|}}{\text{C-NH}_2}}$$
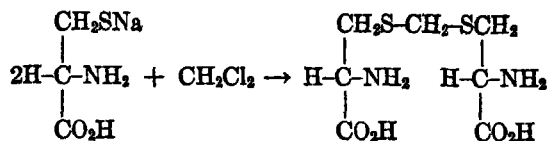
S-Aryl derivatives of cysteine have been prepared by the action of diazo compounds upon cysteine;[293, 289] on acetylation they are converted into mercapturic acids. Bromobenzene, given by mouth to a dog, is excreted in the urine as $l(-)$-p-bromophenylmercapturic acid,[294]

$$\underset{\underset{\text{CO}_2\text{H}}{|}}{\overset{\overset{\text{CH}_2\text{-SC}_6\text{H}_4\text{Br}}{|}}{\text{H-C-NHCOCH}_3}}$$
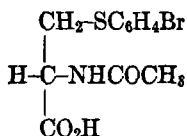
in unstable combination with some unknown compound, possibly a glycuronic acid. The extent to which the bromobenzene is converted to the mercapturic acid depends largely upon the amount of cystine available in the body.[295]

$l(+)$-Cysteine reacts with aqueous formaldehyde to yield $l(-)$- thiazolidinecarboxylic acid [296]

$$\underset{\underset{\text{CO}_2\text{H}}{|}}{\overset{\overset{\text{CH}_2\text{---S}}{|}}{\text{H-C---NH}}} \diagdown_{\text{CH}_2}$$
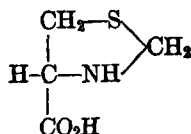
in which the basic properties are markedly weaker than in cysteine.

Like serine, cysteine and cystine break down in hot alkaline solution to yield ammonia and pyruvic acid; sulfide is also formed. The decomposition is accelerated by the presence of lead oxide, pyruvate, or aromatic aldehydes; acylation also increases the instability towards alkali. Nicolet, from a consideration of the behavior of β-ketonic sulfides toward alkali,

$$\text{RCO-CH}_2\text{-CHR-SR} \rightleftarrows \text{RC(OH)=CH-CHR-SR} \rightleftarrows \text{RCO-CH=CHR} + \text{RSH}$$
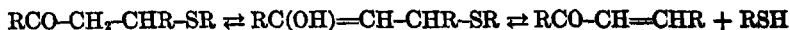
[291] van Veen and Hyman, *Rec. trav. chim.*, **54**, 493 (1935).

[292] du Vigneaud and Patterson, *J. Biol. Chem.*, **114**, 533 (1936).

[293] Friedmann, *Beitr. chem. Physiol. Path.*, **4**, 486 (1904).

[294] Baumann and Preusse, *Ber.*, **12**, 806 (1879); *Z. physiol. Chem.*, **5**, 309 (1881); Jaffé, *Ber.*, **12**, 1092 (1879).

[295] Kapfhammer, *Z. physiol. Chem.*, **116**, 302 (1921); Muldoon, Shiple, and Sherwin, *J. Biol. Chem.*, **59**, 675 (1924).

[296] Ratner and Clarke, *J. Am. Chem. Soc.*, **59**, 200 (1937).

has suggested [297] that the alkaline decomposition of cysteine takes the following course:

$$\underset{\underset{NH_2}{|}}{\overset{\overset{SH}{|}}{CH_2}}-CH-\overset{\overset{O}{||}}{C}OH \rightleftarrows \underset{\underset{NH_2}{|}}{\overset{\overset{SH}{|}}{CH_2}}-C=\overset{\overset{OH}{|}}{C}OH \rightleftarrows H_2S + CH_2=\underset{\underset{NH_2}{|}}{\overset{\overset{O}{||}}{C}}-\overset{}{C}OH \rightarrow CH_3CO-CO_2H + NH_3$$
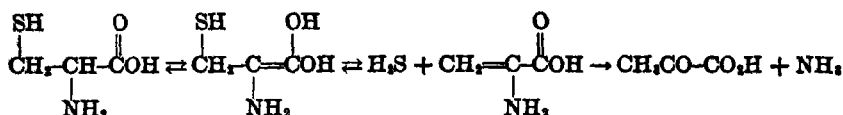
supporting this view by the synthesis of cysteine derivatives from mercaptans and unsaturated azlactones or the corresponding open-chain esters.

$$\underset{\underset{\displaystyle CO\text{——}O}{|\quad\quad|}}{C_6H_5CH=C-N=CC_6H_5} + C_7H_7SH + EtONa$$

$$\searrow \underset{\underset{\displaystyle C_7H_7S\quad CO_2Et}{|\quad\quad|}}{C_6H_5CH-CH-NHCOC_6H_5}$$

$$\underset{\underset{\displaystyle CO_2Et}{|}}{C_6H_5CH=C-NHCOC_6H_5} \nearrow$$

$$+ C_7H_7SH$$
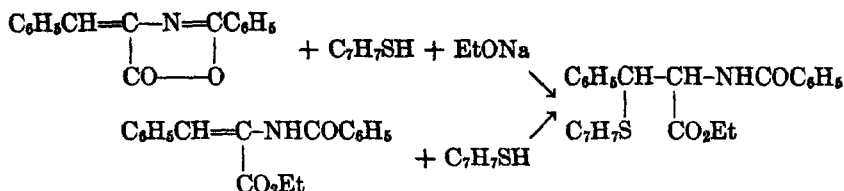
Methionine. Although it had for many years been evident that sulfur must exist in proteins in a form other than that of cystine or cysteine, the first definite hint of its nature was afforded in 1914 by the observation of Mörner [298] that oxidation of proteins with nitric acid leads to the formation of methanesulfonic acid in yields totally unrelated to their content of cystine. That this compound is not formed from pure cystine was shown by direct experiment. In 1923 Mueller [299] isolated from protein hydrolysates a crystalline amino acid isomeric with ethylcysteine but differing from it in being stable to boiling alkali. The same product was shortly thereafter obtained from yeast by extraction with 80 per cent alcohol. Its constitution was established by Barger and Coyne,[300] who synthesized the racemic form by applying the Strecker synthesis to β-methylthiolpropionaldehyde.

$$CH_3SCH_2-CH_2-CHO + HCN + NH_3 \overset{HCl}{\longrightarrow} CH_3SCH_2-CH_2-CH(NH_2)-CO_2H$$
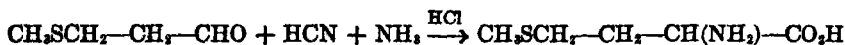
More advantageous syntheses, later developed, involve the application of the malonic ester [301] and the phthaliminomalonic ester [302] procedures to β-chloroethyl methyl sulfide.

Methionine (levorotatory in water) is now recognized as an almost universal constituent of proteins, from which it is liberated by acid hy-

[297] Nicolet, ibid., **53**, 3066 (1931); **54**, 1998 (1932); J. Biol. Chem., **95**, 389 (1932).
[298] Mörner, Z. physiol. Chem., **93**, 175 (1914).
[299] Mueller, J. Biol. Chem., **56**, 156 (1923).
[300] Barger and Coyne, Biochem. J., **22**, 1417 (1928).
[301] Windus and Marvel, J. Am. Chem. Soc., **52**, 2575 (1930).
[302] Barger and Weichselbaum, Biochem. J., **25**, 997 (1931).

drolysis and by the action of proteolytic enzymes.[303] Like other mono-amino monocarboxylic acids, it is extractable from neutral solution by butyl alcohol; it is a frequent contaminant of leucine of protein origin,[304] from which it can be separated as a sparingly soluble mercury derivative.[305]

Methionine stimulates the growth of rats on diets from which cystine is absent.[306] Its biochemical conversion into cystine [307] has been proved by the demonstration that radioactive sulfur, when fed to rats in the form of methionine, appears in the tissue proteins as radioactive cystine,[308] the sulfur atom of which does not exchange with hydrogen sulfide [309] in aqueous solution.

In hot 60 per cent sulfuric acid methionine is converted into homocystine

$$S-CH_2-CH_2-CH(NH_2)-CO_2H$$
$$|$$
$$S-CH_2-CH_2-CH(NH_2)-CO_2H$$
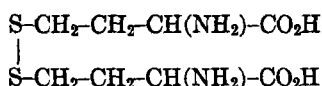
with only slight racemization,[310] and in boiling hydriodic acid it breaks down into methyl iodide and the thiolactone of homocysteine,

$$\begin{array}{ccc} CH_2-CH_2-CHNH_2 & & CH_2-CH_2-CHNH_2 \\ | \qquad\qquad | & + HI \rightarrow CH_3I + & | \qquad\qquad | \\ CH_3S \qquad CO_2H & & S\text{------}CO \end{array}$$
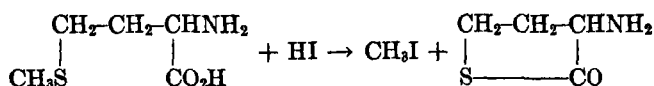
a reaction which forms the basis for an analytical method for the estimation of methionine.[311]

Homocystine has been synthesized [312] by the malonic ester method. During the early stages of the synthesis, the sulfur atom is protected by a benzyl group, which is later removed (as α,β-diphenylethane and toluene) by the action of sodium in liquid ammonia.

$$C_6H_5CH_2S-CH_2-CH_2Cl+NaCH(CO_2Et)_2 \rightarrow C_6H_5CH_2S-CH_2-CH_2-CH(CO_2Et)_2 \rightarrow$$

$$C_6H_5CH_2S-CH_2-CH_2-CH(CO_2H)_2 \xrightarrow{Br_2} C_6H_5CH_2S-CH_2-CH_2-CBr(CO_2H)_2 \xrightarrow{NH_3}$$

$$C_6H_5CH_2S-CH_2-CH_2-CH(NH_2)-CO_2H \xrightarrow{Na} HSCH_2-CH_2-CH(NH_2)-CO_2H \xrightarrow{O_2}$$

$$[-SCH_2-CH_2-CH(NH_2)-CO_2H]_2$$
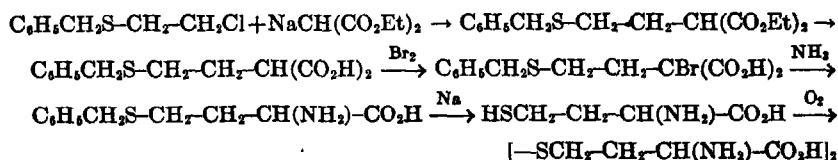
[303] Pirie, *ibid.*, **26**, 1270, 2041 (1932); **27**, 202 (1933).

[304] Mueller, *Science*, **81**, 50 (1935).

[305] Hill and Robson, *Biochem. J.*, **28**, 1008 (1934).

[306] Jackson and Block, *J. Biol. Chem.*, **98**, 465 (1932); du Vigneaud, Dyer, and Harmon, *ibid.*, **101**, 719 (1933).

[307] White and Lewis, *ibid.*, **98**, 607 (1932); Brand, Cahill, and Harris, *ibid.*, **109**, 69 (1935).

[308] Tarver and Schmidt, *ibid.*, **130**, 67 (1939).

[309] Tuck, *J. Chem. Soc.*, 1292 (1939).

[310] du Vigneaud and collaborators, *J. Biol. Chem.*, **99**, 135 (1932); **109**, 97 (1935).

[311] Baernstein, *ibid.*, **106**, 451 (1934); **115**, 24 (1936).

[312] Patterson and du Vigneaud, *ibid.*, **111**, 393 (1935).

The homocysteine, which in the last step is oxidized to homocystine, is stable only in alkaline solution; in the presence of acid it loses water to form the thiolactone,[313] which has no reducing action on iodine until the ring has been opened by alkali. In hot 60 per cent sulfuric acid homocysteine undergoes oxidation to homocystine to a greater extent than ring closure—a finding which partially explains the formation of homocystine from methionine. Cold or more dilute sulfuric acid favors thiolactonization.

Homocystine can be converted into methionine by successively treating it in liquid ammonia solution with sodium and methyl iodide.[306] By resolving S-benzylhomocysteine into its optical components and subjecting the products to the reactions outlined above, it has been possible to prepare the active isomers of homocystine and methionine.[314]

The higher homologs, homomethionine ($n = 3$), pentocystine ($n = 3$), hexomethionine ($n = 4$), and hexocystine ($n = 4$),

$$CH_3S(CH_2)_nCH(NH_2)CO_2H \qquad [-S(CH_2)_nCH(NH_2)CO_2H]_2$$

have been prepared by analogous methods. Unlike the preceding members of the series, none of the four is utilizable for growth in place of cystine.[315]

An amino acid to which the constitution

$$HO_2C-CH(NH_2)-CH_2-S-CH_2-CH_2-CH(NH_2)-CO_2H$$
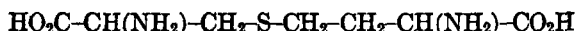
is ascribed has been isolated from the products of the action of sodium sulfide on wool.[316] This compound, which contains the structures of both cysteine and methionine, may be merely an artifact and not a true protein component.

Lysine. The classical methods devised by Kossel and Kutscher,[317] for the separation of the three basic amino acids or "hexone bases," form the basis for the modern procedures developed by Vickery [318] and Block.[319] An excess of silver sulfate or nitrate is added to a sulfuric acid hydrolysate previously adjusted to pH 3–6 with barium hydroxide, and the acidity is further reduced by gradual addition of more barium

[313] Riegel and du Vigneaud, *ibid.*, **112**, 149 (1935).

[314] du Vigneaud and Patterson, *ibid.*, **109**, 97 (1935).

[315] du Vigneaud and collaborators, *ibid.*, **106**, 401 (1934); **108**, 73 (1935); **120**, 11 (1937).

[316] Küster and Irion, *Z. physiol. Chem.*, **184**, 225 (1929).

[317] Kossel and Kutscher, *ibid.*, **31**, 165 (1900).

[318] Vickery and collaborators, *J. Biol. Chem.*, **75**, 115 (1927); **86**, 107 (1930); **93**, 105 (1931).

[319] Block, *ibid.*, **106**, 457 (1934).

hydroxide. At pH 7.0–7.4 histidine, at pH 13–14 arginine precipitate, as their silver derivatives. Lysine, which remains in the filtrate, is subsequently thrown down as its phosphotungstate and finally converted into the picrate.

$l(+)$-Lysine is a fairly general constituent of proteins, though it is absent, or nearly so, from alcohol-soluble proteins of vegetable origin. In only those proteins which contain lysine can the amino group be detected.[320] The lysine appears to be linked with other amino acids through its carboxyl and α-amino groups, the terminal group supplying practically all the free amino groups ascertainable in proteins by means of nitrous acid,[321] for the values so obtained correspond closely to one-half of those for lysine nitrogen. Moreover, no lysine is obtainable from proteins which have been deaminized by nitrous acid.[322] In the reaction with nitrous acid (Van Slyke procedure) evolution of nitrogen from proteins can be almost completely suppressed by chilling to 0–3°; at this temperature lysine yields only one-half of its nitrogen, but at 32° yields all in 5 minutes. It is the terminal group of lysine which is the less reactive, for alanine responds readily and completely to nitrous acid at 3°.[323] Proteins which have been benzoylated or benzenesulfonylated yield on hydrolysis the ε-benzoyl [324] and ε-benzenesulfonyl [325] derivatives of lysine, respectively.

dl-Lysine has been synthesized by reducing ethyl α-isonitroso-δ-cyanovalerate with sodium and alcohol; [326]

$$\text{NC–CH}_2\text{–CH}_2\text{–CH}_2\text{Cl} + \text{NaCH(CO}_2\text{Et)}_2 \rightarrow \text{NC–CH}_2\text{–CH}_2\text{–CH}_2\text{–CH(CO}_2\text{Et)}_2$$

$$\xrightarrow{\text{EtONO}} \text{NC–CH}_2\text{–CH}_2\text{–CH}_2\text{–C(=NOH)–CO}_2\text{Et}$$

$$\xrightarrow{\text{Na,EtOH}} \text{NH}_2\text{CH}_2\text{–CH}_2\text{–CH}_2\text{–CH}_2\text{–CH(NH}_2\text{)–CO}_2\text{H}$$
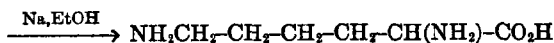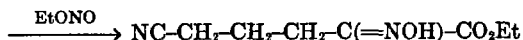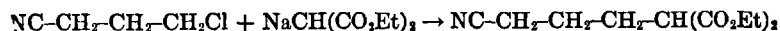
by reduction of the condensation product of γ-chlorobutyronitrile and sodium ethyl phthaliminomalonate, followed by hydrolysis; [327] and from benzoylpiperidine [328] by the following steps:

[320] Kossel and collaborators, Z. physiol. Chem., **76**, 457; **81**, 274 (1912).

[321] Van Slyke and Birchard, J. Biol. Chem., **16**, 539 (1914).

[322] Skraup and Kaas, Ann., **351**, 379 (1906); Kossel and Weiss, Z. physiol. Chem., **78**, 402 (1912).

[323] Sure and Hart, J. Biol. Chem., **31**, 527 (1917).

[324] Goldschmidt and Kinsky, Z. physiol. Chem., **183**, 244 (1929).

[325] Gurin and Clarke, J. Biol. Chem., **107**, 395 (1934).

[326] Fischer and Weigert, Ber., **35**, 3772 (1902).

[327] Sörensen, Compt. rend. trav. lab. Carlsberg, **6**, 1 (1903) [Chem. Zentr., (II), **33** (1903)]

[328] v. Braun, Ber., **42**, 839 (1909).

$$
\begin{array}{c}
\text{CH}_2\text{-CH}_2\text{-N-COC}_6\text{H}_5 \\
| \qquad\qquad | \\
\text{CH}_2\text{-CH}_2\text{-CH}_2
\end{array}
\xrightarrow{\text{PCl}_5}
\begin{array}{c}
\text{CH}_2\text{-CH}_2\text{Cl} \\
| \\
\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-NHCOC}_6\text{H}_5
\end{array}
\xrightarrow{\text{KCN}}
$$

$$
\begin{array}{c}
\text{CH}_2\text{-CH}_2\text{-CN} \\
| \\
\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-NHCOC}_6\text{H}_5
\end{array}
\xrightarrow{\text{KOH}}
\begin{array}{c}
\text{CH}_2\text{-CH}_2\text{-CO}_2\text{H} \\
| \\
\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-NHCOC}_6\text{H}_5
\end{array}
\xrightarrow{\text{Br}_2}
$$

$$
\begin{array}{c}
\text{CH}_2\text{-CHBr-CO}_2\text{H} \\
| \\
\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-NHCOC}_6\text{H}_5
\end{array}
\xrightarrow{\text{NH}_3}
\begin{array}{c}
\text{CH}_2\text{-CH(NH}_2)\text{-CO}_2\text{H} \\
| \\
\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-NHCOC}_6\text{H}_5
\end{array}
\xrightarrow{\text{HCl}}
$$

$$
\begin{array}{c}
\text{CH}_2\text{-CH(NH}_2)\text{-CO}_2\text{H} \\
| \\
\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-NH}_2
\end{array}
$$
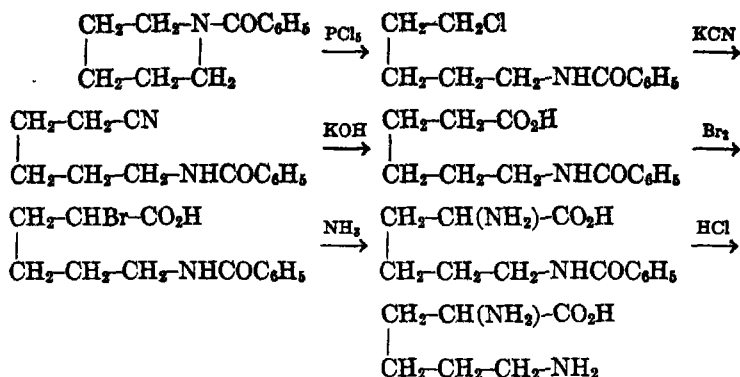
In a convenient modification [329] of this synthesis, the intermediate ε-benzoylaminocaproic acid is prepared from cyclohexanoneoxime.

$$
\begin{array}{c}
\text{CH}_2\text{-CH}_2\text{-C=NOH} \\
| \qquad\qquad | \\
\text{CH}_2\text{-CH}_2\text{-CH}_2
\end{array}
\xrightarrow{\text{H}_2\text{SO}_4}
\begin{array}{c}
\text{CH}_2\text{---CH}_2\text{---CO} \\
| \qquad\qquad\qquad | \\
\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-NH}
\end{array}
\xrightarrow{\text{dil H}_2\text{SO}_4}
$$

$$
\begin{array}{c}
\text{CH}_2\text{-CH}_2\text{-CO}_2\text{H} \\
| \\
\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-NH}_2
\end{array}
\xrightarrow{\text{C}_6\text{H}_5\text{COCl}}
\begin{array}{c}
\text{CH}_2\text{-CH}_2\text{-CO}_2\text{H} \\
| \\
\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-NHCOC}_6\text{H}_5
\end{array}
$$
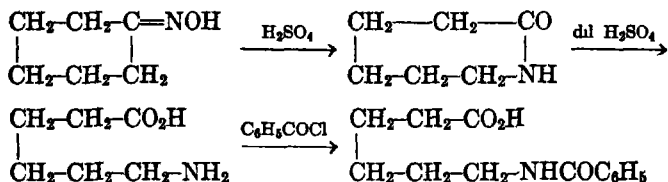
Lysine forms a dibenzoyl derivative, lysuric acid,[330] which on partial hydrolysis by acid yields a monobenzoyl compound.[326] On treatment with nitrosyl bromide this is converted into α-bromo-ε-benzoylaminocaproic acid identical with that obtained from benzoylpiperidine, and with phenyl isocyanate it yields a phenylureide readily convertible into a phenylhydantoin.[331] It must therefore be the ε-monobenzoyllysine:

$$
\begin{array}{c}
\text{CH}_2\text{NHCOC}_6\text{H}_5 \\
| \\
(\text{CH}_2)_3 \\
| \\
\text{CHNHCOC}_6\text{H}_5 \\
| \\
\text{CO}_2\text{H}
\end{array}
\xrightarrow{\text{HCl}}
\begin{array}{c}
\text{CH}_2\text{NHCOC}_6\text{H}_5 \\
| \\
(\text{CH}_2)_3 \\
| \\
\text{CHNH}_2 \\
| \\
\text{CO}_2\text{H}
\end{array}
\xrightarrow{\text{NOBr}}
\begin{array}{c}
\text{CH}_2\text{NHCOC}_6\text{H}_5 \\
| \\
(\text{CH}_2)_3 \\
| \\
\text{CHBr} \\
| \\
\text{CO}_2\text{H}
\end{array}
$$

$$\downarrow \text{C}_6\text{H}_5\text{NCO}$$

$$
\begin{array}{c}
\text{CH}_2\text{NHCOC}_6\text{H}_5 \\
| \\
(\text{CH}_2)_3 \\
| \\
\text{CHNHCONHC}_6\text{H}_5 \\
| \\
\text{CO}_2\text{H}
\end{array}
\xrightarrow{\text{HCl}}
\begin{array}{c}
\text{CH}_2\text{NHCOC}_6\text{H}_5 \\
| \\
(\text{CH}_2)_3 \\
| \\
\text{CH---NH} \\
| \qquad\qquad \searrow\text{CO} \\
\text{CO-N(C}_6\text{H}_5)\nearrow
\end{array}
$$
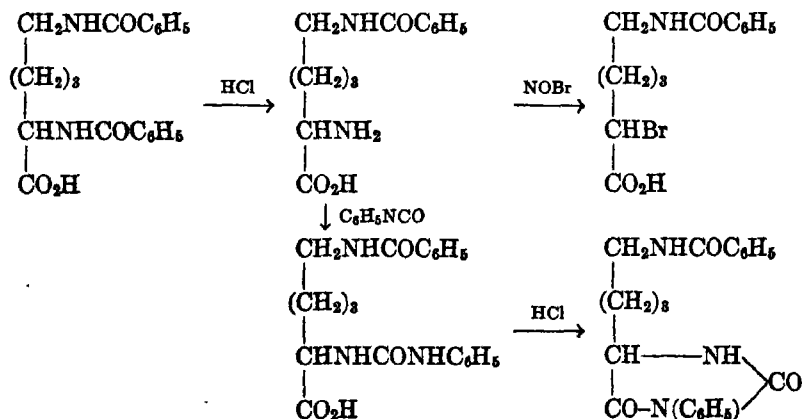
[329] Eck and Marvel, *J. Biol. Chem.*, **106**, 387 (1934).
[330] Drechsel, *Ber.*, **28**, 3189 (1895).
[331] Karrer and Ehrenstein, *Helv. Chim. Acta*, **9**, 323 (1926).

Benzoylated proteins, on hydrolysis, yield the same monobenzoyl lysine, the constitution of which has been confirmed by oxidation to δ-benzoyl-aminovaleric acid.[324] Alkaline hydrolysis of lysuric acid likewise causes the removal of the α-benzoyl group.[332] On the other hand, dibenzenesulfonyl lysine on acid hydrolysis loses a benzenesulfonyl group exclusively from the ϵ-position.[325]

Unnatural $d(-)$-lysine is nutritionally ineffective (p. 1084), probably because of the metabolic unavailability of some intermediary form such as

$$CH_2\text{-}CH_2\text{-}C\text{-}CO_2H$$
$$CH_2\text{-}CH_2\text{-}N$$
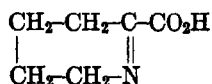
produced by the internal condensation of the terminal amino group with the α-keto group formed by the oxidative deamination which is a normal catabolic process.

Evidence for the existence of a new diamino acid in gelatin has recently been adduced.[333] Its chemical composition is that of a hydroxylysine; as on oxidation with alkaline periodate it (like serine) yields formaldehyde and ammonia,[334] hydroxyl and amino groups occupy neighboring positions at the end of a chain. Two possible structures are proposed:

$$CH_2(NH_2)CHOHCH_2CH_2CH(NH_2)CO_2H$$

or

$$CH_2OHCH(NH_2)CH_2CH_2CH_2(NH_2)CO_2H$$

$l(+)$-**Arginine** appears to be a universal component of proteins, from which it is partially split off by enzymes during the early stages of proteolysis.[335] It is particularly abundant in the protamines of fish sperm; these highly basic polypeptides contain up to 90 per cent of their nitrogen in the form of arginine.[317, 336] Its properties are largely determined by the presence of the strongly basic (p. 1112) guanidino group. There is a marked contrast between its titration curve and that of lysine [337] in the region of pH above 10. When amino acids are titrated in 85 per cent

[332] Karrer and Ehrenstein, ibid., **9**, 1063 (1926).

[333] Van Slyke, Hiller, Dillon, and MacFadyen, Proc. Soc. Exptl. Biol. Med., **38**, 548 (1938).

[334] Van Slyke, Hiller, MacFadyen, Hastings, and Klemperer, J. Biol. Chem. **133**, 287 (1940).

[335] Dauphinee and Hunter, Biochem. J., **24**, 1128 (1930); Lieben and Lieber, Biochem. Z., **275**, 38 (1934).

[336] Kossel and Dakin, Z. physiol. Chem., **41**, 407 (1904); Taylor, J. Biol. Chem., **5**, 389 (1909).

[337] Schmidt, Kirk, and Appleman, ibid., **88**, 285 (1930).

alcohol, or in the presence of formalin, amino groups lose their basic function (*cf.* p. 1090); the guanidino group does not.[28, 338]

The guanidino group in proteins is selectively decomposed by sodium hypochlorite or hot alkalies.[339] The products, which yield but little arginine on hydrolysis, are no longer soluble in dilute acids and are not digestible by pepsin, but are readily hydrolyzed by trypsin. When sodium hypochlorite is added to an alkaline solution of arginine with α-naphthol, a red color is produced. This, the Sakaguchi, test is positive with proteins containing combined arginine [340] and with monosubstituted guanidines such as methylguanidine or glycocyamine; it is also given by *sym*-dimethylguanidine and *sym*-trimethylguanidine but not by asymmetrically di- and trisubstituted guanidines (e.g., *as*-dimethylguanidine, glycocyamidine, creatine) nor guanidine itself.[341] It has been developed into a quantitative procedure for the estimation of arginine.[342] Proteins also give the color, in intensities equivalent to their content of combined arginine.

Arginine, in common with other guanidino derivatives containing two hydrogen atoms attached to a single nitrogen atom, develops a violet color on treatment with biacetyl or acetyl benzoyl in alkaline solution.[343] This color reaction, which has been placed on a quantitative basis, depends upon the following series of reactions:

$$\begin{array}{ccc} \overset{O\ O}{\underset{\parallel\ \parallel}{CH_2\text{-}C\text{-}C\text{-}R}} & \overset{O\ O}{\underset{\parallel\ \parallel}{CH\text{-}C\text{-}C\text{-}R}} & \overset{R_2N\text{-}C(=NH)\text{-}N\ \ N\text{-}C(=NH)\text{-}NR_2}{\underset{\parallel}{CH\text{-}C\text{-}C\text{-}R}} \\ \underset{\overset{\parallel\ \parallel}{O\ O}}{R\text{-}C\text{-}C\text{-}CH_3} \to & \underset{\overset{\parallel}{O}}{R\text{-}C\text{-}C\text{-}CH_3} \to & \underset{\overset{\parallel}{N\text{-}C(=NH)\text{-}NR_2}}{RC\text{-}C\text{-}CH_3} \end{array}$$
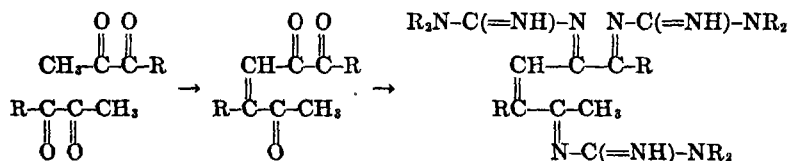
Creatine (p. 1111) responds to this test; creatinine does not.

Another, and far more specific, method is based upon the hydrolysis of arginine into ornithine and urea under the influence of an enzyme, arginase, present in mammalian liver.[344]

$$NH_2\text{-}C(=NH)\text{-}NH\text{-}(CH_2)_3\text{-}CH(NH_2)\text{-}CO_2H \to$$

$$CO(NH_2)_2 + NH_2\text{-}(CH_2)_3\text{-}CH(NH_2)\text{-}CO_2H$$
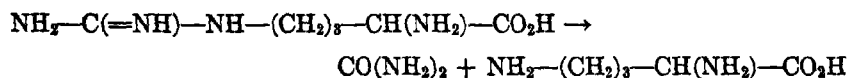
The urea so formed is estimated either as ammonia after hydrolysis by

[338] Levy, *ibid.*, **109**, 365 (1935).

[339] Sakaguchi, *J. Biochem. (Japan)*, **5**, 143, 159 (1925).

[340] Sakaguchi, *ibid.*, **5**, 25, 133 (1925).

[341] Poller, *Ber.*, **59**, 1927 (1926).

[342] Weber, *J. Biol. Chem.*, **86**, 217; **88**, 353 (1930); Jorpes and Thorén, *Biochem. J.*, **26**, 1504 (1932).

[343] Harden and Norris, *J. Physiol.*, **42**, 332 (1911); Lang, *Z. physiol. Chem.*, **208**, 273 (1932).

[344] Kossel and Dakin, *ibid.*, **41**, 321; **42**, 181 (1904).

urease [345] or directly by treatment with xanthydrol,[346] which causes the quantitative precipitation of dixanthylurea.[347]

$$CO\left[NH\text{—}CH\underset{C_6H_4}{\overset{C_6H_4}{\diagdown}}O\right]_2$$
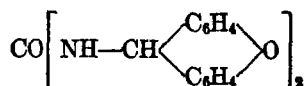
The conversion of arginine to ornithine and urea can also be effected by boiling with barium hydroxide;[348] on boiling with 20 per cent (or stronger) sodium hydroxide,[349] arginine is converted into ornithine and two equivalents of ammonia.

The method of separating arginine from other amino acids by its precipitation at high alkalinity as a silver derivative analogous to the insoluble compound $CH_3N_3Ag_2 \cdot H_2O$ formed by guanidine under similar conditions has been supplemented, for analytical and preparative purposes, by a process based on the observation that arginine forms a sparingly soluble salt with 2,4-dinitro-1-naphthol-7-sulfonic (flavianic) acid.[350, 318] The flavianate may conveniently be converted, by means of strong hydrochloric acid, in which the free flavianic acid is not freely soluble, into the very soluble dihydrochloride; this yields the crystalline monohydrochloride on treatment with aniline.[351] For analytical purposes, arginine may be completely precipitated as the diflavianate from an acid hydrolysate of a protein by the addition of 4 to 5 moles of flavianic acid per mole of arginine; this diflavianate is then dissolved in ammonia and quantitatively precipitated by acidification.[352]

Arginine forms an insoluble benzylidene derivative which separates selectively when protein hydrolysates are rendered strongly alkaline and treated with benzaldehyde.[80, 353] Benzylidene arginine is readily decomposed into benzaldehyde and a salt of arginine on warming with dilute mineral acid. The inability of this compound to form a sodium salt is attributable to the failure of the alkali metal to replace the equally strongly basic guanidino group present in the molecule. A similar effect is observed with the hydantoic acid formed by the action of potassium cyanate upon arginine monohydrochloride:[354] the solubility of this product in water is not increased by the addition of alkali.

[345] Hunter and Dauphinee, J. Biol. Chem., **85**, 627 (1930).
[346] Bonot and Cahn, Compt. rend., **184**, 246 (1927).
[347] Fosse, ibid., **158**, 1076 (1914); Ann. chim., [9] **6**, 13 (1916).
[348] Schulze and collaborators, Ber., **24**, 2701 (1891); **30**, 2879 (1897).
[349] Van Slyke, J. Biol. Chem., **10**, 15 (1911); Plimmer, Biochem. J., **10**, 115 (1916).
[350] Kossel and Gross, Z. physiol. Chem., **135**, 167 (1924).
[351] Cox, J. Biol. Chem., **78**, 475 (1928).
[352] Vickery, ibid., **132**, 325 (1940).
[353] Brand and Sandberg, "Organic Syntheses," John Wiley & Sons, New York (1932), Vol. 12, p. 4.
[354] Boon and Robson. Biochem. J., **29**, 2573 (1935).

On treatment with sodium nitrite and acetic acid in the Van Slyke procedure, arginine gives rise to only one molecule of nitrogen;[355] although guanidine reacts rapidly with nitrous acid in the presence of mineral acid, it does not do so in acetic acid,[356] and the guanidino group in arginine behaves in the same way. Proteins, on hydrolysis after treatment with sodium nitrite and acetic acid, yield arginine but no lysine.[357]

The guanidino group is resistant to the action of barium permanganate, by which arginine is oxidized first to γ-guanidinobutyric acid [358] and finally to guanidine.[359] Proteins yield guanidine on similar treatment.[360]

Like guanidine, arginine can be nitrated.[361] Nitroarginine, which can also be obtained by hydrolysis of a nitrated protamine, lacks the strongly basic character of arginine, which is regenerated by catalytic hydrogenation.[362]

$$NO_2-NH-C(=NH)-NH-(CH_2)_3-CH(NH_2)-CO_2H \xrightarrow{H}$$

$$NH_3 + NH_2-C(=NH)NH-(CH_2)_3CH(NH_2)-CO_2H$$
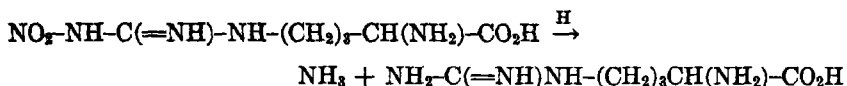
This reaction has been adapted to meet the needs of peptide synthesis.

An analogous compound, arginine-phosphoric acid, has been isolated from the muscle of marine invertebrates, in which it plays the part taken by creatine-phosphoric acid (p. 1113) in muscular contraction of vertebrates.[363] Arginine-phosphoric acid

$$CO_2H-CH(NH_2)-(CH_2)_3-NH-C(=NH)-NH-PO(OH)_2$$

in which the dissociation constant of the guanidino group has been lowered by the introduction of the phosphoryl group, is hydrolyzed to arginine and phosphoric acid during muscular activity and is resynthesized during relaxation.

Acyl derivatives of arginine can be prepared by the usual methods, the α-amino group being far more readily acylated than the guanidino group. With benzoyl chloride and alkali, a dibenzoyl derivative is formed;[364] on the other hand, in attempts to prepare the corresponding

[355] Van Slyke, *J. Biol. Chem.*, **9**, 185 (1911); Plimmer, *Biochem. J.*, **18**, 105 (1924); Hunter, *J. Biol. Chem.*, **83**, 731 (1929).

[356] Hynd and Macfarlane, *Biochem. J.*, **20**, 1264 (1926).

[357] Traxl, *Monatsh.*, **29**, 59 (1908).

[358] Kutscher, *Z. physiol. Chem.*, **32**, 413 (1901).

[359] Bénech and Kutscher, *ibid.*, **32**, 278 (1901).

[360] Lossen, *Ann.*, **201**, 369 (1880); Otori, *Z. physiol. Chem.*, **43**, 86 (1904); Kutscher and Schenck, *Ber.*, **38**, 455 (1905).

[361] Kossel and Kennaway, *Z. physiol. Chem.*, **72**, 486 (1911).

[362] Bergmann, Zervas, and Rinke, *ibid.*, **224**, 40 (1934).

[363] Meyerhof and Lohmann, *Biochem. Z.*, **196**, 22, 49 (1928); Riesser and Hansen, *Z. physiol. Chem.*, **219**, 62 (1933).

[364] Gulewitsch, *ibid.*, **27**, 178 (1899).

di-β-naphthalenesulfonyl derivative [365] only one acyl group could be introduced. On treatment with benzenesulfonyl chloride in presence of potassium carbonate, arginine forms the α-monobenzenesulfonyl derivative, but a second benzenesulfonyl group may be introduced by the use of concentrated sodium hydroxide in excess.[366]

With acetic anhydride in the cold, arginine yields a monoacetyl derivative, which has become racemized under the combined influence of the excess of anhydride and the strongly polar guanidino group (cf. p. 1094). Boiling acetic anhydride leads to the production of a triacetyl anhydroarginine,[367] which on treatment with water breaks down into diacetylurea and β-acetamino-α-piperidone.

$$
\begin{array}{cc}
\text{NH-(CH}_2)_3\text{-CH-NH}_2 & \\
\text{C=NH} \quad \text{CO}_2\text{H} & \xrightarrow{\text{Ac}_2\text{O}} \\
\text{NH}_2 &
\end{array}
\quad
\begin{array}{c}
\text{NH-(CH}_2)_3\text{-CH-NHAc} \\
\text{C=NH} \quad \text{CO}_2\text{H} \quad \xrightarrow{\text{Ac}_2\text{O}} \\
\text{NH}_2
\end{array}
$$

$$
\begin{array}{c}
\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-CH-NHAc} \\
\text{N}\text{————————CO} \\
\text{C=NAc} \\
\text{NHAc}
\end{array}
\xrightarrow{\text{H}_2\text{O}}
\text{CO(NHAc)}_2 +
\begin{array}{c}
\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-CH-NHAc} \\
\text{NH}\text{————————CO}
\end{array}
$$
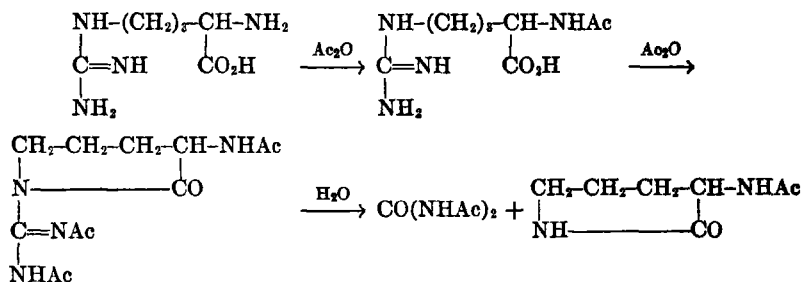
The piperidone derivative, when boiled with acids, readily undergoes hydrolysis to ornithine. The triacetyl anhydroarginine reacts not only with water, but with amines, which are thereby converted into derivatives of guanidine.

When the methyl ester of arginine is liberated from its hydrochloride, it undergoes auto-condensation,[368] yielding ornithine ester and an anhydride of α,δ-diguanidinovaleric acid [369] containing a glycocyamidine group. The reaction, which is analogous to that involved in the synthesis of glycocyamidine from guanidine and glycine ester (p. 1111), is explained as consisting in the condensation of the ester group of one molecule with the guanidino group of another, followed immediately by disproportionate cleavage.

$$2\text{NH}_2\text{-C(=NH)-NH-(CH}_2)_3\text{-CH(NH}_2)\text{-CO}_2\text{CH}_3 \rightarrow$$

$$
\text{NH}_2\text{-C(=NH)NH(CH}_2)_3\text{CH(NH}_2)\text{-C-NH-C(=NH)NH(CH}_2)_3\text{CH(NH}_2)\text{-CO}_2\text{CH}_3 \rightarrow
$$
$$\overset{\|}{\text{O}}$$

$$
\begin{array}{c}
\text{NH}_2\text{-C(=NH)-NH-(CH}_2)_3\text{-CH————————CO} \\
\text{NH-C(=NH)-NH}
\end{array}
+ \text{NH}_2\text{-(CH}_2)_3\text{-CH(NH}_2)\text{-CO}_2\text{CH}_3
$$
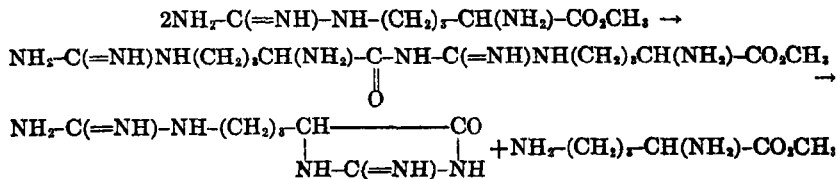
[365] Riesser, ibid., **49**, 210 (1906).
[366] Clarke and Gillespie, J. Am. Chem. Soc., **54**, 1964 (1932).
[367] Bergmann and Köster, Z. physiol. Chem., **159**, 179 (1926).
[368] Fischer and Suzuki, Ber., **38**, 4173 (1905).
[369] Zervas and Bergmann, Ber., **61**, 1195 (1928).

Natural $l(+)$-arginine has the same configuration as the other natural amino acids.[17] The levorotatory variety is not attacked by arginase and may therefore conveniently be prepared by the action of liver press-juice upon dl-arginine.[365] On the other hand, arginase readily hydrolyses γ-guanidinobutyric acid to γ-aminobutyric acid and urea, but is without action on ε-guanidinocaproic acid.[370] The kinetics of arginase action is imperfectly understood, for although hydrolysis is partially inhibited by ornithine, addition of the other end product, urea, is without effect.[371]

Ornithine does not appear to be a constituent of proteins. However, its dibenzoyl derivative, ornithuric acid, occurs in the excreta of birds receiving benzoic acid in their diet.[372] Hippuric acid, the principal form in which benzoic acid is eliminated by mammals, is not produced by chickens under these conditions, even when glycine is administered simultaneously.[373] Similarly, the difuroyl derivative of ornithine is excreted by chickens receiving furfural, which gives rise to furoylglycine in rabbits and dogs.[374]

On alkaline hydrolysis, ornithuric acid loses the terminal benzoyl group. The l-α-monobenzoylornithine so prepared from natural arginine, on successive treatment with nitrous acid and hot hydriodic acid, is converted into l-proline.[331]

$$
\begin{array}{ccccc}
CH_2NH\text{-}C(=NH)\text{-}NH_2 & & CH_2NH_2 & & CH_2\text{-}NHCOC_6H_5 \\
| & & | & & | \\
CH_2 & & CH_2 & & CH_2 \\
| & \rightarrow & | & \rightarrow & | & \rightarrow \\
CH_2 & & CH_2 & & CH_2 \\
| & & | & & | \\
H\text{—}C\text{—}NH_2 & & H\text{—}C\text{—}NH_2 & & H\text{—}C\text{—}NHCOC_6H_5 \\
| & & | & & | \\
CO_2H & & CO_2H & & CO_2H
\end{array}
$$

$$
\begin{array}{ccccc}
CH_2NH_2 & & CH_2OH & & CH_2\text{—} \\
| & & | & & | \\
CH_2 & & CH_2 & & CH_2 \\
| & \rightarrow & | & \rightarrow & | \\
CH_2 & & CH_2 & & CH_2 \\
| & & | & & | \\
H\text{—}C\text{—}NHCOC_6H_5 & & H\text{—}C\text{—}NHCOC_6H_5 & & H\text{—}C\text{——}NH \\
| & & | & & | \\
CO_2H & & CO_2H & & CO_2H
\end{array}
$$
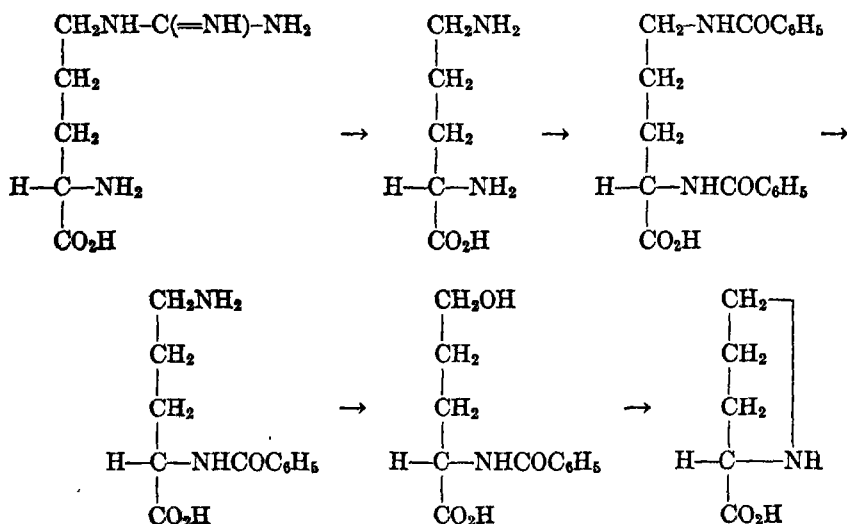
[370] Thomas, Z. physiol. Chem., 88, 465 (1913).

[371] Gross, ibid., 112, 236 (1921).

[372] Jaffé, Ber., 10, 1925 (1877); Ellinger, Z. physiol. Chem., 29, 334 (1900).

[373] Yoshikawa, ibid., 68, 79 (1910).

[374] Jaffé and Cohn, Ber., 20, 2311 (1887); 21, 3461 (1888).

This series of reactions confirms the configurational identity of natural arginine, ornithine, and proline.

Ornithine has been synthesized by several general methods already discussed. The rather unstable free base has recently been obtained in a crystalline condition.[375] Its properties closely resemble those of lysine, but the picrate is more soluble in water.[376] It is converted into arginine by the addition of cyanamide.[377] The methyl ester, when liberated from its hydrochloride, undergoes autocondensation, not to a diketopiperazine but to β-amino-α-piperidone,[378]

$$CH_2\text{-}CH(NH_2)\text{-}CO_2CH_3 \qquad CH_2\text{-}CH(NH_2)\text{-}CO$$
$$\begin{array}{ccc} | & \rightarrow & | \qquad\qquad | \\ CH_2\text{-}CH_2\text{-}NH_2 & & CH_2\text{---}CH_2\text{---}NH \end{array}$$
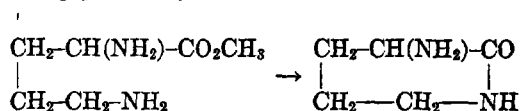
from which ornithine is regenerated on heating with hydrochloric acid. dl-Citrulline, isolated from watermelon juice in 1914 by Koga and Odake,[379] was recognized as α-amino-δ-carbamidovaleric acid by Wada,[380] who confirmed its constitution by synthesis. It has been isolated from a tryptic digest of casein,[381] and is readily formed,[382] as the sparingly soluble copper salt, by heating ornithine copper sulfate with aqueous urea:

$$NH_2CONH_2 + NH_2(CH_2)_3CH(NH_2)CO_2H \rightarrow$$

$$NH_2CONH(CH_2)_3CH(NH_2)CO_2H + NH_3$$
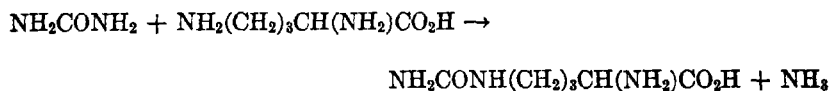
The hydrolysis of arginine to citrulline is conveniently accomplished [383] by boiling with an equimolar quantity of alkali in 2.8 N solution. Complete loss of optical activity occurs during the process.

The relationship between arginine, ornithine, and citrulline is of extreme importance in the production of urea in the living body. Krebs and Henseleit [384] have shown that the synthesis of urea, in the presence of intact liver tissue, from ammonia and the carbon dioxide resulting from the biochemical oxidation of glucose, lactic acid, or pyruvic acid, is markedly accelerated by the addition of any one of the above three

[375] Vickery and Cook, J. Biol. Chem., 94, 393 (1931).
[376] Kossel and Weiss, Z. physiol. Chem., 68, 160 (1910).
[377] Schulze and Winterstein, Ber., 32, 3191 (1899); Z. physiol. Chem., 34, 128 (1901)
[378] Fischer and Zemplén, Ber., 42, 4878 (1909).
[379] Koga and Odake, J. Chem. Soc. Japan, 35, 519 (1914).
[380] Wada, Biochem. Z., 224, 420 (1930).
[381] Wada, ibid., 257, 1 (1933).
[382] Kurtz, J. Biol. Chem., 122, 477 (1938).
[383] Fox, ibid., 123, 687 (1938).
[384] Krebs and Henseleit, Z. physiol. Chem., 210, 33 (1932).

amino acids. The process may be schematically expressed as follows:

$$CO_2 + NH_3 + Ornithine \rightarrow Citrulline$$

$$NH_3 + Citrulline \rightarrow Arginine + H_2O$$

$$Arginine \rightarrow Ornithine + CO(NH_2)_2$$
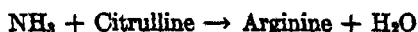
The first and second reactions take place through the influence of some enzyme present in surviving liver tissue, for no urea synthesis occurs if the tissue is crushed; the third reaction is, of course, brought about by arginase.

The administration to a mouse of ornithine containing deuterium in the carbon chain results in its conversion to arginine, for much of the deuterium reappears in the arginine obtained by hydrolysis of the proteins of the animal.[385] At the same time some degradation of the ornithine occurs, as deuterium is also found in the glutamic acid and proline of the proteins.[386] The glutamic acid is no doubt formed by oxidation of the terminal aminomethyl group to carboxyl; the formation of proline is plausibly explainable by the following series of biochemical reactions:
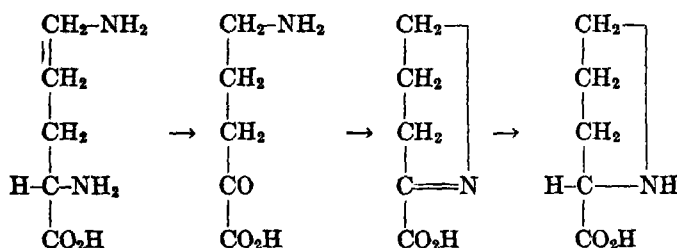
In octopine, a constituent of muscle of scallops and other marine invertebrates, a molecule of arginine and one of alanine share a single α-imino nitrogen atom.[387, 388]
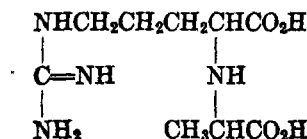
It has been synthesized from l-α-bromopropionic acid and l-arginine;[387] mixtures of diastereoisomers have been obtained from ethyl dl-α-bromo-

[385] Clutton, Schoenheimer, and Rittenberg, *J. Biol. Chem.*, **132**, 227 (1940).

[386] Roloff, Ratner, and Schoenheimer, *ibid.*, **136**, 561 (1940).

[387] Akasi, *J. Biochem. (Japan)*, **25**, 261, 281, 291 (1937); **26**, 129 (1937).

[388] Moore and Wilson, *J. Biol. Chem.*, **119**, 573 (1937).

propionate and l-arginine methyl ester [389] and by the catalytic hydrogenation of a mixture of l-arginine and pyruvic acid. [390]

$$
\begin{array}{ccc}
\underset{\underset{\displaystyle CO_2H}{|}}{\overset{\displaystyle R}{\underset{|}{HC-NH_2}}} + \underset{\underset{\displaystyle CO_2H}{|}}{\overset{\displaystyle CH_3}{\underset{|}{CO}}} & \rightarrow \underset{\underset{\displaystyle CO_2H}{|}}{\overset{\displaystyle R}{\underset{|}{HC-NH-}}}\underset{\underset{\displaystyle CO_2H}{|}}{\overset{\displaystyle CH_3}{\underset{|}{COH}}} & \rightarrow \underset{\underset{\displaystyle CO_2H}{|}}{\overset{\displaystyle R}{\underset{|}{HC-NH-}}}\underset{\underset{\displaystyle CO_2H}{|}}{\overset{\displaystyle CH_3}{\underset{|}{CH}}}
\end{array}
$$
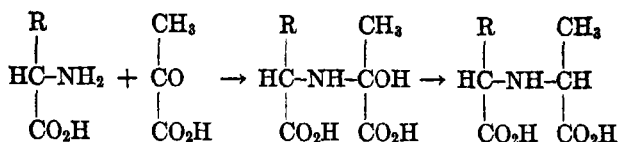
The enzyme urease, which promotes the hydrolysis of urea and is widely employed for its estimation and, indirectly, that of arginine (p. 1142), is usually secured from jack bean meal. Aqueous-alcoholic extracts of this bean contain a basic amino acid, canavanine, $C_5H_{12}O_3N_4$, which, like arginine, yields half of its nitrogen as urea on treatment with liver extract. This amino acid [391] responds to the ninhydrin test (p. 1099) and gives up only one of its nitrogen atoms to nitrous acid in the Van Slyke procedure; it is therefore an α-amino acid. It is more stable than arginine towards barium hydroxide, forms a tribenzoyl derivative, yields no guanidine on oxidation with barium permanganate, and responds to neither the Sakaguchi (p. 1142) nor the biacetyl (p. 1142) test. It gives a characteristic red color with a solution of sodium nitroprusside which has been autoxidized by exposure to air and light.

Canaline, $C_4H_{10}O_3N_2$, formed together with urea by the action of an enzyme (canavanase) present in liver extract, contains one α-amino group and one nitrogen atom which does not react with nitrous acid. It forms a dibenzoyl derivative. It gives a red color with alkaline picrate (Jaffé test, p. 1114) but none with autoxidized nitroprusside. On catalytic reduction it is converted into ammonia and α-amino-γ-hydroxybutyric acid; [392] this points to the constitution:

$$NH_2O-CH_2-CH_2-CH(NH_2)-CO_2H$$

The reduction of the oxygen-nitrogen bond is analogous to that brought about by hydriodic acid with α-benzylhydroxylamine.[393] Canavanine and dibenzoylcanaline do not respond to catalytic hydrogenation. On the other hand, by the action of hydrobromic acid canavanine yields α-amino-γ-bromobutyric acid and guanidine.[394]

$$NH_2-C(=NH)-NH-O-CH_2-CH_2-CH(NH_2)-CO_2H \xrightarrow{\text{HBr}}$$

$$NH_2C(=NH)-NH_2 + Br-CH_2-CH_2-CH(NH_2)-CO_2H$$
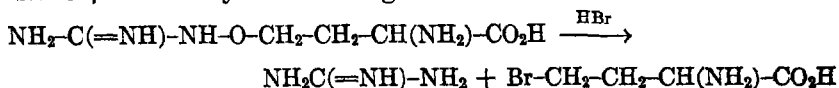
[389] Irvin and Wilson, ibid., 127, 555 (1939).

[390] Knoop and Martins, Z. physiol. Chem., 258, 238 (1939).

[391] Kitagawa and collaborators, J. Biochem. (Japan), 11, 265 (1929); 16, 339 (1932); 18, 333 (1933); 23, 181; 24, 407 (1936); [C. A., 28, 2678 (1934); 29, 7280 (1935)].

[392] Fischer and Blumenthal, Ber., 40, 106 (1907); Sörensen and Andersen, Z. physiol Chem., 56, 250 (1908).

[393] Meyer, Ber., 16, 167 (1883).

[394] Gulland and Morris, J. Chem. Soc., 763 (1935).

Canaline has been regenerated from α-amino-γ-hydroxybutyric acid by condensing the γ-iodo-α-benzoylamino acid with benzhydroxamic acid.

$$HO-CH_2-CH_2-CH(NH_2)-CO_2H \rightarrow HO-CH_2-CH_2-CH(NHCOC_6H_5)-CO_2H \xrightarrow{HCl}$$

$$\begin{matrix} CH_2-CH_2-CH-NHCOC_6H_5 \\ | \qquad\qquad | \\ O\text{————}CO \end{matrix} \xrightarrow{HI} I-CH_2-CH_2-CH(NHCOC_6H_5)-CO_2H$$

$$\xrightarrow{C_6H_5CO-NHOH} C_6H_5CO-NHO-CH_2-CH_2-CH(NHCOC_6H_5)-CO_2H \xrightarrow{HCl}$$

$$NH_2O-CH_2-CH_2-CH(NH_2)-CO_2H$$
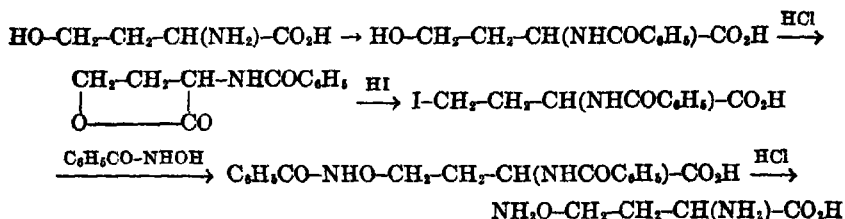
The reconversion of canaline into canavanine has been effected by the use of methylisourea.

$$NH_2O-(CH_2)_2-CH(NH_2)-CO_2H \xrightarrow{C_6H_5COCl}$$

$$C_6H_5CONHO-(CH_2)_2-CH(NHCOC_6H_5)-CO_2H \xrightarrow{HCl}$$

$$NH_2O-(CH_2)_2-CH(NHCOC_6H_5)-CO_2H \xrightarrow{NH_2-C(=NH)-OCH_3}$$

$$NH_2-C(=NH)-NHO-(CH_2)_2-CH(NHCOC_6H_5)-CO_2H \xrightarrow{HCl}$$

$$NH_2-C(=NH)-NHO-(CH_2)_2-CH(NH_2)-CO_2H$$
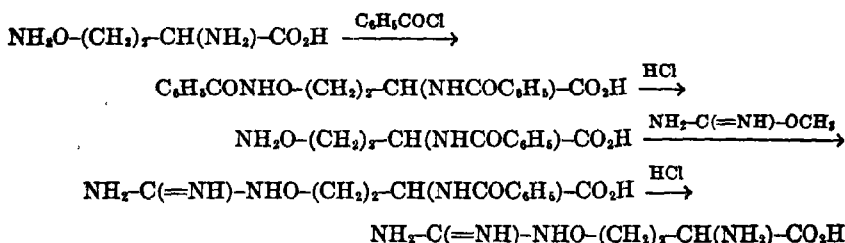
As is to be expected of an O-ether of hydroxylamine, canaline can form condensation products with aldehydes. An ethylidene derivative, which can be prepared from canaline and acetaldehyde, has been isolated from the products of the digestion of canavanine with liver extract.

Canavanine appears to be the first derivative of hydroxyguanidine to have been studied since that substance was originally synthesized.[395] Its titration curve [396] indicates, by reference to corresponding data for other compounds,[397] that the guanidoxyl ($NH_2C(=NH)NHO-$) group, in contrast to the guanidine group, is a weaker base than ammonia. The aminoxyl (NH_2O-) group, present in canaline, is even more weakly basic than the amino group of aniline or the imidazole of histidine.

When boiled in neutral solution, canavanine loses ammonia with formation of desaminocanavanine.[398]

$$\begin{matrix} O-CH_2-CH_2 \\ | \qquad\qquad\quad \diagdown \\ \qquad\qquad\qquad\quad CH-CO_2H \\ NH-C-NH \diagup \\ \quad \| \\ \quad NH \end{matrix}$$
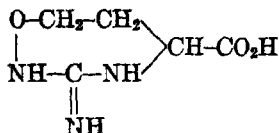
[395] Prätorius-Seidler, *J. prakt. Chem.*, [2] **21**, 129 (1880).
[396] Tomiyama, *J. Biol. Chem.*, **111**, 45 (1935).
[397] Borek and Clarke, *ibid.*, **125**, 479 (1938).
[398] Tsukamoto, *J. Biochem. (Japan)*, **26**, 373 (1937).

From a study of analogous compounds, it appears that the color with autoxidized nitroprusside is characteristic of the guanidoxyl, that with picrate of the aminoxyl, group.

$l(+)$-**Histidine**, an almost universal constituent of proteins, is dextrorotatory in acid solution, but levorotatory in water and in alkali. It was discovered simultaneously by Kossel [399] in the fraction precipitated by mercuric chloride from the hydrolysate of a protamine of fish roe, and by Hedin [400] in the salt $C_6H_7O_2N_2Ag_2 \cdot H_2O$ precipitated from protein hydrolysates on adding silver nitrate and alkali. Both processes have subsequently been developed into convenient preparative methods; the former is applicable when only histidine is desired,[401] the latter when the preparation of all the "hexone bases" is necessary.[402] Mercuric sulfate in 5 per cent sulfuric acid (Hopkins's reagent) has been recommended [403] for the estimation of histidine in small samples of protein.

Nitranilic acid, which forms a very sparingly soluble salt

$$C_6O_2(OH)_2(NO_2)_2 \cdot C_6H_9O_2N_2$$

with histidine,[146] has also been proposed [404] as an analytical precipitant.

For preparative purposes, histidine is conveniently obtained from blood corpuscle paste by acid hydrolysis and precipitation of histidine with mercuric chloride.[405]

The imidazole (glyoxaline) group in histidine is a relatively weak base; the dihydrochloride, when dissolved in water, hydrolyzes to the monohydrochloride,[406] and the free amino acid, unlike lysine and arginine, is extracted by butyl alcohol from aqueous solution at pH 7,[23] together with the monoamino monocarboxylic acids. Histidine forms well-defined salts with one and with two molecules of picrolonic acid; [407] the diflavianate is sparingly soluble and of use for isolation, but the monoflavianate is difficult to prepare; [408] it also forms a sparingly soluble Reineckate [409] which crystallizes out with those of proline and hydroxyproline (pp. 1118, 1125). It is precipitated from acid solution by phosphotungstic acid but tends to redissolve in excess of the precipitant.[410]

[399] Kossel, Z. physiol. Chem., **22**, 176 (1896).

[400] Hedin, ibid., **22**, 191 (1896).

[401] Hanke and Koessler, J. Biol. Chem., **43**, 521 (1920).

[402] Vickery and Leavenworth, ibid., **78**, 627 (1928).

[403] Rosedale and da Silva, Biochem. J., **26**, 369 (1932).

[404] Block, J. Biol. Chem., **133**, 67 (1940).

[405] Foster and Shemin, "Organic Syntheses," John Wiley & Sons, New York (1938) Vol. 18, p. 43.

[406] Abderhalden and Einbeck, Z. physiol. Chem., **62**, 322 (1909).

[407] Brigl, ibid., **64**, 337 (1910).

[408] Vickery, J. Biol. Chem., **71**, 303 (1926).

[409] Kapfhammer and Spörer, Z. physiol. Chem., **173**, 245 (1928).

[410] Fränkel, Monatsh., **24**, 229 (1903).

The constitution of histidine was established by Pauly,[394] whose discovery that it yields an azo color with diazobenzenesulfonic acid forms the basis of a widely employed method for its colorimetric estimation.[411] The imidazole nucleus accepts either one [412] or two [413] azo groups. The chief interfering substance in a protein hydrolysate is tyrosine; the ability of this to couple can be inhibited by benzoylation, which under suitable conditions is without effect on the Pauly reaction for histidine.[414] Histidine also survives treatment with nitric acid, which nitrates tyrosine and so prevents it from coupling; [415] this procedure, however, suffers from the disadvantage of introducing a yellow color into the mixture to be tested. The most satisfactory procedure is to apply the Pauly test to a fraction previously precipitated by phosphotungstic acid or by silver.

A red color is formed on adding bromine water to a solution of histidine and changes to purple on addition of ammonia. This color reaction has been developed for the quantitative estimation of histidine, for which it appears to be specific.[416] A transient red-violet color is developed on treating histidine with hydrogen peroxide in the presence of a ferrous salt,[417] presumably with the intermediate formation of β-imidazoleacetaldehyde.

Histidine has been synthesized [418] from the sodium derivative of ethyl chloromalonate and 4-chloromethylimidazole, prepared from α,γ-diaminoacetone,

$$
\begin{array}{ccccccccc}
CH_2\text{-}CO_2H & & CH\!=\!NOH & & CH_2NH_2 & & CH\!-\!N & & \\
| & & | & & | & & \parallel\quad\diagdown & & \\
CO & \xrightarrow{HNO_2} & CO & \xrightarrow{SnCl_2} & CO & \xrightarrow{KSCN} & \quad\quad C\text{-}SH & \xrightarrow{HNO_2} & \\
| & & | & & | & & C\!-\!NH & & \\
CH_2\text{-}CO_2H & & CH\!=\!NOH & & CH_2NH_2 & & | & & \\
& & & & & & CH_2NH_2 & &
\end{array}
$$

$$
\begin{array}{ccccc}
CH\!-\!N & & CH\!-\!N & & CH\!-\!N \\
\parallel\quad\diagdown & & \parallel\quad\diagdown & & \parallel\quad\diagdown \\
\quad\quad CH & \rightarrow & \quad\quad CH & \rightarrow & \quad\quad CH \\
C\!-\!NH & & C\!-\!NH & & C\!-\!NH \\
| & & | & & | \\
CH_2NH_2 & & CH_2OH & & CH_2Cl
\end{array}
$$
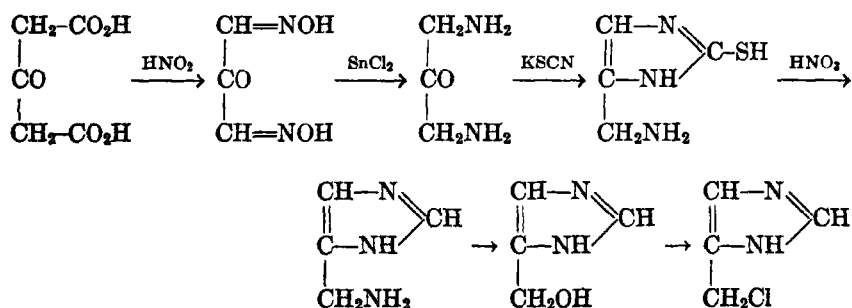
[411] Jorpes, *Biochem. J.*, **26**, 1507 (1932).

[412] Wallach, Ruhg, and Behrend, *Ann.*, **271**, 28 (1892).

[413] Pauly, *Z. physiol. Chem.*, **94**, 284 (1915).

[414] Inouye, *ibid.*, **88**, 79 (1913).

[415] Brunswik, *ibid.*, **127**, 268 (1923).

[416] Knoop, *Beitr. chem. Physiol. Path.*, **11**, 356 (1908); Kapeller-Adler, *Biochem. Z.*, **264**, 121 (1933).

[417] Kikkoji and Neuberg, *Biochem. Z.*, **20**, 523 (1909).

[418] Pyman, *J. Chem. Soc.*, **99**, 668, 1386 (1911); **109**, 186 (1916).

$$
\begin{array}{c}
\text{CH—N} \\
\|\quad\diagdown\!\!\diagup\text{CH} \\
\text{C—NH} \\
| \\
\text{CH}_2\text{Cl}
\end{array}
\;+\; \text{NaCCl(CO}_2\text{Et)}_2 \;\rightarrow\;
\begin{array}{c}
\text{CH—N} \\
\|\quad\diagdown\!\!\diagup\text{CH} \\
\text{C—NH} \\
| \\
\text{CH}_2\text{-CCl(CO}_2\text{Et)}_2
\end{array}
\;\rightarrow
$$

$$
\begin{array}{c}
\text{CH—N} \\
\|\quad\diagdown\!\!\diagup\text{CH} \\
\text{C—NH} \\
| \\
\text{CH}_2\text{-CHCl-CO}_2\text{H}
\end{array}
\qquad\rightarrow\;
\begin{array}{c}
\text{CH—N} \\
\|\quad\diagdown\!\!\diagup\text{CH} \\
\text{C—NH} \\
| \\
\text{CH}_2\text{-CH(NH}_2\text{)-CO}_2\text{H}
\end{array}
$$
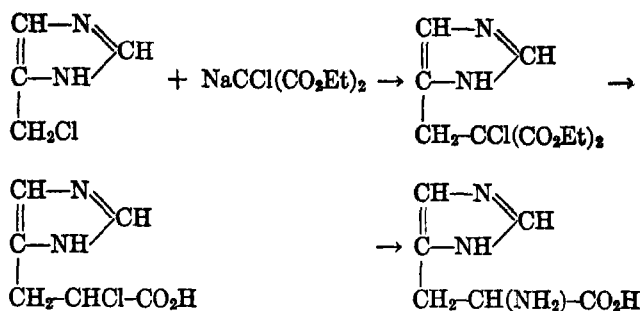
The racemic histidine was resolved by crystallizing the d-tartrate. It has also been synthesized by the Erlenmeyer process from imidazolealdehyde and hippuric acid.

Two of the nuclear hydrogen atoms of histidine are replaceable by iodine, when the α-amino group is previously protected by benzoylation.[419]

The α-monobenzoyl derivative is formed with one molecular proportion of benzoyl chloride in presence of benzene and the minimally practicable amount of aqueous alkali; a benzoyl group can be introduced into the imidazole nucleus [420] by treating the methyl ester of α-benzoyl-histidine in benzene solution with a semi-molecular proportion of benzoyl chloride.

$$
2\;
\begin{array}{c}
\text{CH—N} \\
\|\quad\diagdown\!\!\diagup\text{CH} \\
\text{C—N} \\
| \qquad\diagdown\text{H} \\
\text{CH}_2\text{-CH(NHCOC}_6\text{H}_5\text{)-CO}_2\text{CH}_3
\end{array}
\qquad +\; \text{C}_6\text{H}_5\text{COCl} \;\rightarrow
$$

$$
\begin{array}{c}
\text{CH—N} \\
\|\quad\diagdown\!\!\diagup\text{CH} \\
\text{C—N} \\
| \qquad\diagdown\text{COC}_6\text{H}_5 \\
\text{CH}_2\text{-CH(NHCOC}_6\text{H}_5\text{)-CO}_2\text{CH}_3
\end{array}
\quad +\;
\begin{array}{c}
\text{CH—N} \\
\|\quad\diagdown\!\!\diagup\text{CH} \cdot \text{HCl} \\
\text{C—NH} \\
| \\
\text{CH}_2\text{-CH(NHCOC}_6\text{H}_5\text{)-CO}_2\text{CH}_3
\end{array}
$$
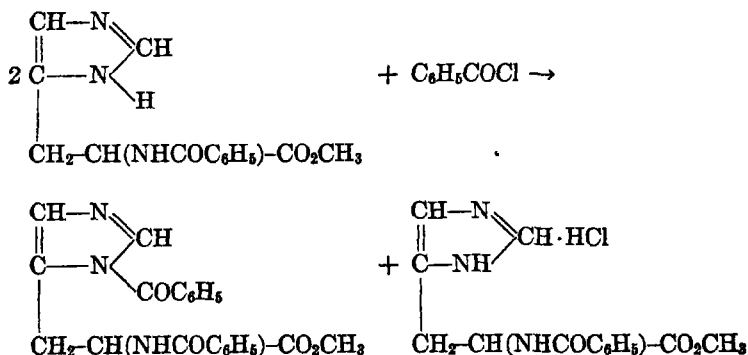
An interesting property of such esters of diacyl histidines is their ability to transfer the nuclear acyl group to amino acids or esters [421] and presumably to other amines. For example, hippuryl chloride reacts with

[419] Pauly, Ber., 43, 2243 (1910).
[420] Gerngross, Z. physiol. Chem., 108, 50 (1919).
[421] Bergmann and Zervas, ibid., 175, 145 (1928).

α-benzoylhistidine methyl ester to form a product which with glycine yields α-benzoylhistidine ester and hippurylglycine.

$$
\begin{array}{l}
\text{N=CH—N-CO–CH}_2\text{–NHCOC}_6\text{H}_5 \\
\qquad | \qquad\quad | \\
\text{CH=\!=\!=C–CH}_2\text{–CH(NHCOC}_6\text{H}_5\text{)–CO}_2\text{CH}_3
\end{array}
\quad + \text{ NH}_2\text{–CH}_2\text{–CO}_2\text{H} \rightarrow
$$

$$
\begin{array}{l}
\text{N=CH-NH} \quad\;\; \text{NHCOC}_6\text{H}_5 \\
\quad | \qquad\quad | \qquad\quad | \\
\text{CH=\!=\!=C–CH}_2\text{–CH–CO}_2\text{CH}_3
\end{array}
\quad + \text{ C}_6\text{H}_5\text{CONH-CH}_2\text{-CO-NH-CH}_2\text{-CO}_2\text{H}
$$
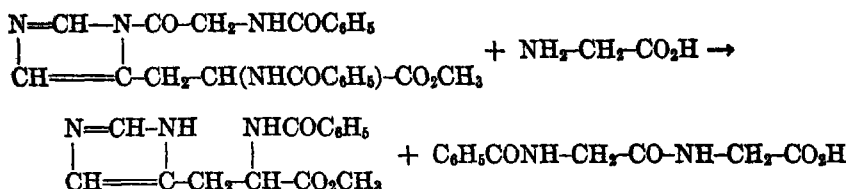
When histidine methyl ester is treated with benzoyl chloride in the presence of aqueous sodium carbonate, the imidazole ring is opened.[422]

$$
\begin{array}{l}
\text{N=CH-NH} \quad\;\; \text{NH}_2 \\
\quad | \qquad\quad | \qquad | \\
\text{CH=\!=\!=C–CH}_2\text{–CH–CO}_2\text{CH}_3
\end{array}
\rightarrow
\begin{array}{l}
\text{C}_6\text{H}_5\text{CONH} \;\; \text{NHCOC}_6\text{H}_5 \;\; \text{NHCOC}_6\text{H}_5 \\
\qquad | \qquad\quad | \qquad\qquad | \\
\text{CH=C–CH}_2\text{———CH-CO}_2\text{CH}_3
\end{array}
$$
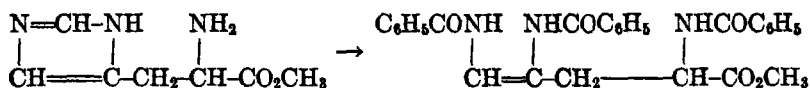
The product on treatment with methyl-alcoholic hydrogen chloride is converted to the methyl ester of γ-keto-ornithuric acid.[423]

$$
\text{C}_6\text{H}_5\text{CONH-CH}_2\text{-CO-CH}_2\text{-CH(NHCOC}_6\text{H}_5\text{)-CO}_2\text{CH}_3
$$
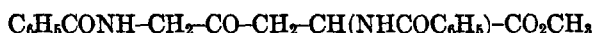
On treatment with ozone, followed by partial hydrolysis, the tribenzoyl compound prepared from natural histidine is converted into the benzoyl derivative of natural $l(-)$-aspartic acid.[424]

$$
\begin{array}{l}
\text{CH-NHCOC}_6\text{H}_5 \\
\;\; \| \\
\text{C-NHCOC}_6\text{H}_5 \\
\;\; | \\
\text{CH}_2 \\
\;\; | \\
\text{H—C—NHCOC}_6\text{H}_5 \\
\;\; | \\
\text{CO}_2\text{CH}_3
\end{array}
\quad \xrightarrow{\text{O}_2} \quad
\begin{array}{l}
\text{CO}_2\text{H} \\
\;\; | \\
\text{CH}_2 \\
\;\; | \\
\text{H—C—NHCOC}_6\text{H}_5 \\
\;\; | \\
\text{CO}_2\text{H}
\end{array}
$$
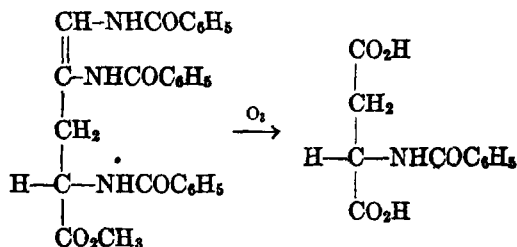
Histidine of protein origin therefore possesses the same configuration as the other natural amino acids, as is also indicated by pH-dependence curves.[17]

On treatment with nitrous acid, histidine yields β-imidazolelactic acid, which is reduced by phosphorus with hydriodic acid to β-imidazole-propionic acid,[425] identical with that prepared from β-glyoxylpropionic acid with formaldehyde and ammonia.

[422] Knoel and Edlbacher, *ibid.*, **93**, 396 (1915).
[423] Langenbeck and Hutschenreuter, *ibid.*, **182**, 305 (1929).
[424] Langenbeck, *Ber.*, **58**, 227 (1925).
[425] Knoop and Windaus, *Beitr. chem. Physiol. Path.*, **7**, 144 (1906).

$$
\begin{array}{c}
\underset{|}{\text{CHO}} \\
\underset{|}{\text{CO}} \\
\underset{|}{\text{CH}_2} \\
\text{CH}_2\text{-CO}_2\text{H}
\end{array}
+ 2\text{NH}_3 + \text{CH}_2\text{O} \rightarrow
\begin{array}{c}
\text{CH-N} \\ \text{C-NH} \\ \underset{|}{\text{CH}_2} \\ \text{CH}_2\text{-CO}_2\text{H}
\end{array}\!\!\!\text{CH}
\leftarrow
\begin{array}{c}
\text{CH-N} \\ \text{C-NH} \\ \underset{|}{\text{CH}_2} \\ \text{CHOH-CO}_2\text{H}
\end{array}\!\!\!\text{CH}
\leftarrow
\begin{array}{c}
\text{CH-N} \\ \text{C-NH} \\ \underset{|}{\text{CH}_2} \\ \text{CH(NH}_2)\text{-CO}_2\text{H}
\end{array}\!\!\!\text{CH}
$$
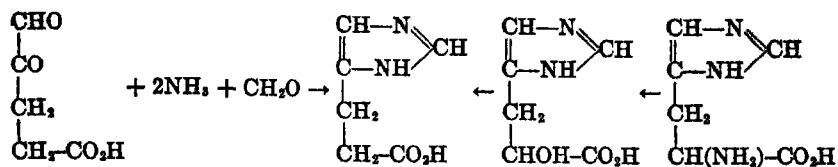
β-Imidazolelactic acid, on oxidation by nitric acid followed by hydrogen peroxide, is successively converted into imidazoleglyoxylic acid and imidazolecarboxylic acid,[426] a series of reactions which served to confirm the assignment of the primary amino group to the α position.

Histidine is converted into β-imidazolelactic acid by some bacteria; others, particularly certain of the *B. coli* group, cause decarboxylation to histamine,[427] a conversion which seems also to be brought about by ultra-violet light. A fungus, *Oidium lactis*, brings about reductive deamination to β-imidazolepropionic acid,[428] and certain microorganisms (e.g., *B. paratyphosus* B) effect deamination without reduction to urocanic acid.[429] This last product, identified by Hunter[430] as β-imidazoleacrylic acid, is a form in which orally administered histidine is eliminated by dogs.[431] However, since less than half of the histidine can be recovered from the urine as imidazole derivatives,[432] some profound decomposition must take place in the body. A reaction of this type is brought about by an enzyme, histidase, present in the liver. Under its influence *l*-histidine breaks down into $l(+)$-glutamic acid and two moles of ammonia,[433] a hydrolytic converse of the classical synthesis of imidazoles from glyoxals, formaldehyde, and ammonia. Indirect evidence points to the intermediate formation of a formylated glutamine.

$$
\begin{array}{c}
\text{CH-N} \\ \text{C-NH} \\ \underset{|}{\text{CH}_2}\text{-CH(NH}_2)\text{-CO}_2\text{H}
\end{array}\!\!\!\text{CH}
\rightarrow
\begin{array}{c}
\text{CO-NH-CHO} \\ \underset{|}{\text{CH}_2} \\ \text{CH}_2\text{-CH(NH}_2)\text{-CO}_2\text{H}
\end{array}
\rightarrow
\begin{array}{c}
\text{CO}_2\text{H} \\ \underset{|}{\text{CH}_2} \\ \text{CH}_2\text{-CH(NH}_2)\text{-CO}_2\text{H}
\end{array}
$$
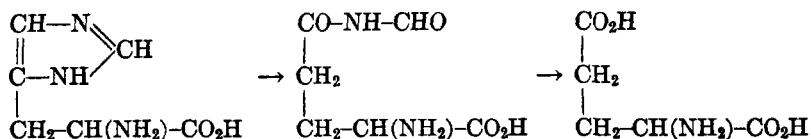
Histidine cannot be synthesized in the mammalian body from simpler compounds, but is apparently so produced from β-imidazolelactic acid and imidazolepyruvic acid, which stimulate growth on diets deficient in

[426] Knoop, *ibid.*, **10**, 111 (1907).

[427] Hanke and Koessler, *J. Biol. Chem.*, **50**, 131 (1922); Hirai, *Biochem. Z.*, **267**, 1 (1933).

[428] Kiyokawa, *Z. physiol. Chem.*, **214**, 38 (1933).

[429] Raistrick, *Biochem. J.*, **11**, 71 (1917).

[430] Hunter, *J. Biol. Chem.*, **11**, 537 (1912).

[431] Kotake and Konishi, *Z. physiol. Chem.*, **122**, 230 (1922).

[432] Abderhalden and Buadse, *ibid.*, **200**, 87 (1931).

[433] Edlbacher and collaborators, *ibid.*, **191**, 225 (1930); **224**, 261 (1934).

histidine but otherwise adequate.[434] Urocanic acid, on the other hand, is almost without effect on growth.

Histamine, the decarboxylation product of histidine, is of great physiological interest, on account of its powerful vasodilator action. It is present in lung, liver, muscle, and blood.[435] Lung and kidney contain a highly specific enzyme, histaminase, which breaks down histamine,[436] much as histidase disrupts *l*-histidine. Histamine has been synthesized by reduction of imidazoleacetonitrile [418] and from α-aminobutyrolactone [392] by the following steps: [437]

$$CH_2-CH_2-CHNH_2 \quad \xrightarrow{NaHg} \quad CH_2-CH_2-CH-NH_2 \quad \xrightarrow{NH_4SCN}$$
$$| \qquad \qquad | \qquad \qquad \qquad | \qquad \qquad |$$
$$O————CO \qquad \qquad O————CHOH$$

$$HOCH_2-CH_2-C-NH \searrow C-SH \xrightarrow{HNO_2} HOCH_2-CH_2-C-NH \searrow CH \xrightarrow{SOCl_2}$$
$$\qquad \qquad || \qquad \qquad \qquad \qquad \qquad \qquad || \qquad$$
$$\qquad \qquad CH-N \diagup \qquad \qquad \qquad \qquad \qquad CH-N \diagup$$

$$Cl-CH_2-CH_2-C-NH \searrow CH \xrightarrow{NH_3} NH_2-CH_2-CH_2-C-NH \searrow CH$$
$$\qquad \qquad || \qquad \qquad \qquad \qquad \qquad || \qquad$$
$$\qquad \qquad CH-N \diagup \qquad \qquad \qquad \qquad CH-N \diagup$$
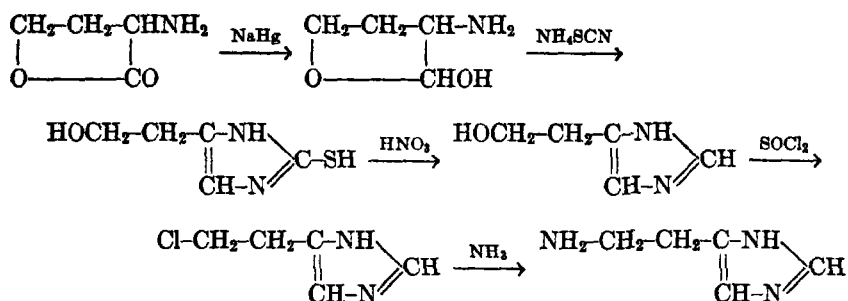
On treatment with benzoyl chloride and alkali the imidazole ring is opened, as in the case of histidine methyl ester; the product, on heating with acid anhydrides and hydrolyzing, yields physiologically inactive 2-alkyl homologs of histamine.[438]

$$CH-NHCOC_6H_5 \cdot \qquad \qquad CH—N \searrow CR \qquad \qquad CH—N \searrow CR$$
$$|| \qquad \qquad \qquad \xrightarrow{(RCO)_2O} \qquad || \qquad \diagup \qquad \qquad || \qquad \diagup$$
$$C-NHCOC_6H_5 \qquad \qquad C—NH \qquad \qquad \rightarrow C—NH$$
$$| \qquad \qquad \qquad \qquad \qquad | \qquad \qquad \qquad \qquad |$$
$$CH_2-CH_2NHCOC_6H_5 \qquad \qquad CH_2-CH_2NHCOR \qquad CH_2-CH_2NH_2$$
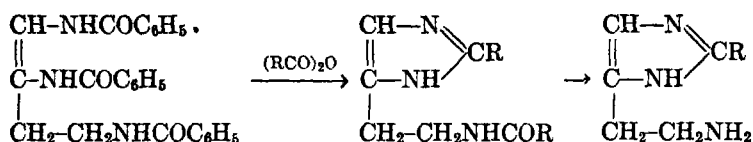
Although 2-thiolhistidine has never been isolated from natural sources, certain proteins, notably zein, give a positive color reaction for thiolimidazoles.[439] Thiolhistidine has been synthesized by the action of thiocyanate upon γ-ketoörnithine, prepared either from histidine (*cf.* p. 1154), or from aspartic acid.[440]

[434] Harrow and Sherwin, *J. Biol. Chem.*, **70**, 683 (1926).

[435] Best, Dale, Dudley, and Thorpe, *J. Physiol.*, **62**, 397 (1926); Thorpe, *Biochem. J.*, **22**, 94 (1928); Barsoum and Gaddum, *J. Physiol.*, **85**, 1 (1935).

[436] McHenry and Gavin, *Biochem. J.*, **26**, 1365 (1932).

[437] Garforth and Pyman, *J. Chem. Soc.*, 489 (1935).

[438] van der Merwe, *Z. physiol. Chem.*, **177**, 301 (1928).

[439] Eagles and Vars, *J. Biol. Chem.*, **80**, 615 (1928).

[440] Ashley and Harington, *J. Chem. Soc.*, 2586 (1930); Harington and Overhoff, *Biochem. J.*, **27**, 338 (1933).

CH$_2$-CO$_2$H
|
CH-NH$_2$ $\xrightarrow{(CH_3CO)_2O}$
|
CO$_2$H

CH$_2$-CO$_2$H
|
CH-N$\diagdown$
| $>$C-CH$_3$
OC—O$\diagup$ $\xrightarrow{PCl_5}$

CH$_2$-COCl
|
CH-N$\diagdown$
| $>$C-CH$_3$
OC—O$\diagup$ $\xrightarrow{CH_2N_2;\ EtOH}$

CH$_2$-CO-CH$_2$Cl
|
CH-NH-COCH$_3$ $\rightarrow$
|
CO$_2$Et

CH$_2$-CO-CH$_2$N$\diagdown$CO$\diagdown$C$_6$H$_4$
| $\diagup$CO$\diagup$
CH-NH-COCH$_3$
| $\xrightarrow{HCl}$
CO$_2$Et

CH$_2$-CO-CH$_2$-NH$_2$
|
CHNH$_2$ $\xrightarrow{NaSCN}$
|
CO$_2$H

CH-N$\diagdown$
‖ $>$C-SH
C-NH$\diagup$
|
CH$_2$
|
CHNH$_2$
|
CO$_2$H
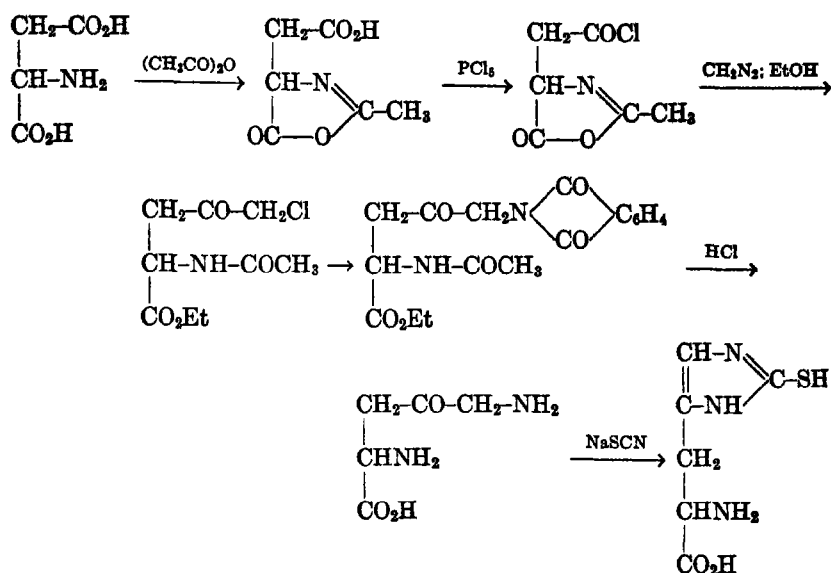
Ergothioneine, the trimethylbetaine of thiolhistidine, is present in ergot and in blood. Attempts to synthesize it by methylating thiolhistidine have failed, owing to the breakdown of the methylated product into trimethylamine and an unsaturated acid. In the same way, histidine trimethylbetaine, formed by oxidizing ergothioneine, breaks down on treatment with alkali [441] into trimethylamine and urocanic acid.

CH-N$\diagdown$
‖ $>$C-SH
C-NH$\diagup$
|
CH$_2$
|
CHN$^+$Me$_3$
|
CO$_2^-$ $\xrightarrow{FeCl_3}$

CH-N$\diagdown$
‖ $>$CH
C-NH$\diagup$
|
CH$_2$
|
CHN$^+$Me$_3$
|
CO$_2^-$ $\xrightarrow{KOH}$

CH-N$\diagdown$
‖ $>$CH
C-NH$\diagup$
|
CH
‖
CH
|
CO$_2$H   + Me$_3$N
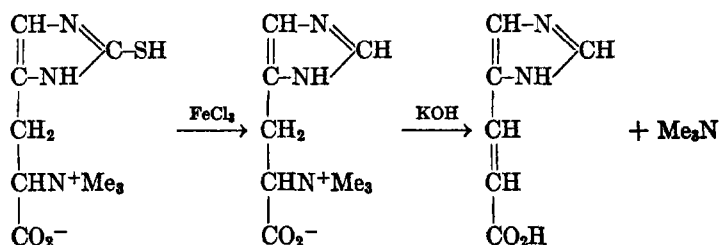
Beef muscle contains a water-soluble base called carnosine [442] which, like histidine, is precipitated by mercuric sulfate and from alkaline solution by silver nitrate, and yields an azo color with diazobenzenesulfonic acid; it gives no color, however, with bromine,[443] and its Reineckate is less soluble than that of histidine.[444] On alkaline hydrolysis it yields

[441] Barger and Ewins, J. Chem. Soc., 99, 2336 (1911).
[442] Gulewitsch and Amiradzibi, Z. physiol. Chem., 30, 565 (1900).
[443] Hunter, Biochem. J., 16, 640 (1922).
[444] Smorodintzev, Biochem. Z., 222, 425 (1930).

l-histidine,[445] and its phenylureide on boiling with acids breaks down into histidine and the phenylureide of β-aminopropionic acid.[446] Carnosine is therefore β-aminopropionylhistidine.

$$NH_2-CH_2-CH_2-CO-NH-CH-CH_2-C\!\!=\!\!=\!\!=\!\!=\!\!CH$$
$$\underset{CO_2H}{|}\qquad\underset{NH-CH=N}{|\qquad\quad|}$$
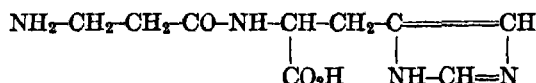
Synthetically it has been obtained, in poor yields, by the action of ammonia upon β-iodopropionylhistidine [447] and by reduction of β-nitropropionylhistidine.[448] A practical synthesis has recently been developed: [449] β-aminopropionic acid is condensed with benzyl chlorocarbonate; the resulting carbobenzoxy-β-alanine is converted successively into its chloride, methyl ester, hydrazide, and azide (*cf.* p. 1107); this is coupled with histidine methyl ester, and, after hydrolysis, the carbobenzoxy group is removed with palladium and hydrogen.

A methyl homolog of carnosine, called anserine,[450] occurs, together with carnosine, in the skeletal muscles of various mammals, fish, reptiles, and birds.[451] It is not precipitated by silver and does not couple with diazo compounds, but is thrown down by mercuric sulfate. On hydrolysis it yields β-alanine and a methylhistidine; [452] on heating with soda-lime it yields 1,5-dimethylimidazole,[453]

$$CH_3-C\!\!=\!\!CH-N$$
$$\underset{N(CH_3)-CH}{|\qquad\quad\|}$$
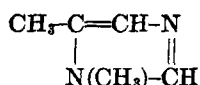
a reaction which establishes the position of the nuclear methyl group. In other respects it closely resembles carnosine, and its properties are in full accord with the structure:

$$NH_2-CH_2-CH_2-CO-NH-CH-CH_2-C\!\!=\!\!=\!\!CH-N$$
$$\underset{CO_2H}{|}\qquad\underset{N(CH_3)-CH}{|\qquad\quad\|}$$
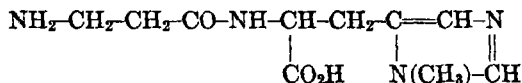
which has been confirmed by a synthesis [454] analogous to that [449] of carnosine.

[445] Gulewitsch, *Z. physiol. Chem.*, **50**, 535 (1907).
[446] Abderhalden and Geidel, *Fermentforschung*, **12**, 518 (1931).
[447] Baumann and Ingvaldsen, *J. Biol. Chem.*, **35**, 263 (1918).
[448] Barger and Tutin, *Biochem. J.*, **12**, 402 (1918).
[449] Sifferd and du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).
[450] Ackermann, Timpe, and Poller, *Z. physiol. Chem.*, **183**, 1 (1929).
[451] Wolff and Wilson, *J. Biol. Chem.*, **109**, 565 (1935).
[452] Linneweh and collaborators, *Z. physiol. Chem.*, **183**, 11 (1929); **189**, 80 (1930).
[453] Keil, *ibid.*, **187**, 1 (1930).
[454] Behrens and du Vigneaud, *J. Biol. Chem.*, **120**, 517 (1937).

l(+)-**Tryptophan** is dextrorotary in strongly acid and in alkaline solution, but highly levorotatory in water. It is a nearly general protein constituent, but is absent, or nearly so, from gelatin, zein, and insulin. It does not appear among the products of acid hydrolysis of proteins, as it is converted by the action of mineral acids into humins, formed by condensation with aldehydes derived from the protein or substances, such as carbohydrates, associated ,with it.[455] Tryptophan is also rapidly destroyed when proteins are hydrolyzed with boiling sodium hydroxide solution, but it is stable to barium hydroxide, which, however, brings about racemization. For the preparation of the natural, optically active product, it is customary to resort to enzymatic hydrolysis by means of trypsin—a slow procedure, which liberates less than half of the tryptophan.[456] The precipitate, characteristic of tryptophan, which forms on addition of mercuric sulfate to the tryptic digest containing 5 per cent of free sulfuric acid [457] contains peptides from which tryptophan is released on further tryptic digestion. Tryptophan is extracted by butyl alcohol from neutral solution; this fact has been usefully applied, for preparative purposes, to the fraction precipitated by mercury.[458]

Natural *l*(+)-tryptophan possesses the same configuration as other amino acids.[17] The racemic variety has been synthesized by the Erlenmeyer procedure from 3-indolealdehyde and hippuric acid.[459]
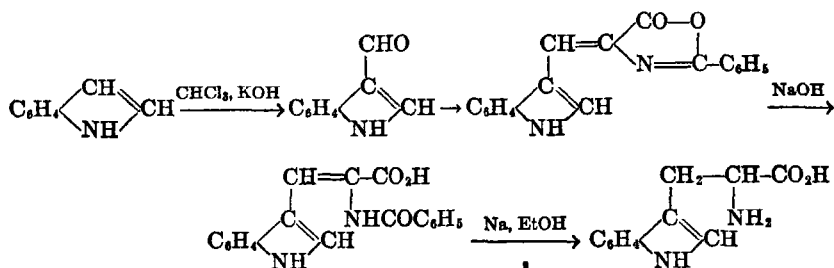
Better yields are obtainable by condensing the aldehyde with hydantoin in presence of piperidine and heating the resulting indolalhydantoin with ammonium sulfide [460] (*cf.* p. 1108).

Colored products are formed on treating tryptophan with aldehydes in presence of concentrated mineral acids. The violet color reaction

[455] Burr and Gortner, *J. Am. Chem. Soc.*, **46**, 1224 (1924).

[456] Onslow, *Biochem. J.*, **15**, 383, 392 (1931).

[457] Hopkins and Cole, *J. Physiol.*, **27**, 418 (1902).

[458] Cox and King, "Organic Syntheses," John Wiley & Sons, New York (1930), Vol. 10, p. 100.

[459] Ellinger and Flamand, *Z. physiol. Chem.*, **55**, 8 (1908).

[460] Boyd and Robson, *Biochem. J.*, **29**, 2256 (1935).

with glyoxylic acid in sulfuric acid, which aided Hopkins and Cole in the discovery of tryptophan, is rendered more certain and sensitive by the addition of a trace of copper.[461] This probably acts as an oxygen carrier, for with other aldehydes the presence of oxidizing agents has been found necessary for the development of a blue color suitable for quantitative purposes. Special use has been made of vanillin,[462] p-dimethylaminobenzaldehyde,[463] and formaldehyde,[464] with hydrogen peroxide and nitrous acid as oxidants. In the absence of oxidants, red to violet tints of indeterminate hue are formed.

It has been suggested [465] that the blue pigments produced from tryptophan with aldehydes and nitrous acid possess the structure:

$$CH_2-CHOH-CO_2H \qquad CH_2-CHOH-CO_2H$$

$$C_6H_4 \underset{N}{\overset{C}{<}} C=\!\!=\!\!C\underset{R}{\overset{}{-}}C \underset{NH}{\overset{C}{>}} C_6H_4$$
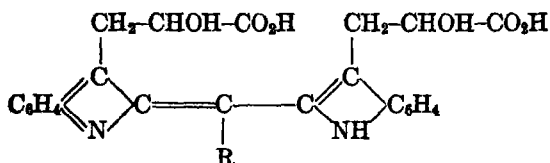
Like tyrosine, tryptophan yields a yellow color with nitric acid [466] and a blue color with Folin's phenol reagent.[237] Both these colorimetric tests have been applied quantitatively, as has the red color, extractable by amyl alcohol, produced with bromine water.[467]

When l-tryptophan is administered to normal rabbits, it is partially eliminated as γ-hydroxyquinoline-α-carboxylic (kynurenic) acid. This compound is also formed, though in lower yields, from indole-3-pyruvic acid.[468] Rabbits on a diet of polished rice, which is deficient in thiamin (vitamin B₁), excrete not only kynurenic acid but also an amino acid, kynurenine, in which the pyrrole ring of tryptophan has ruptured.[469]

$$CH_2-CH(NH_2)-CO_2H \qquad\qquad CH-CH(NH_2)-CO_2H$$

$$C_6H_4 \underset{NH}{\overset{C}{<}} CH \qquad \rightarrow \quad C_6H_4 \underset{NH_2}{\overset{C-CO_2H}{<}}$$
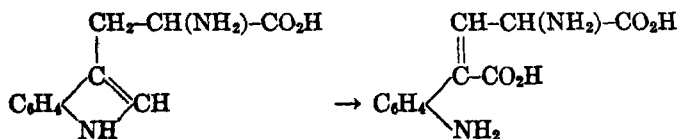
[461] Winkler, Z. physiol. Chem., **228**, 50 (1934).
[462] Ragins, J. Biol. Chem., **80**, 543 (1928).
[463] Boyd, Biochem. J., **23**, 78 (1929).
[464] Fürth and collaborators, Biochem. Z., **109**, 103, 124 (1920).
[465] Ghigi, Gazz. chim. ital., **63**, 411 (1933).
[466] Tillmans, Hirsch, and Stoppel, Biochem. Z., **198**, 379 (1928).
[467] Levene and Rouiller, J. Biol. Chem., **2**, 481 (1907).
[468] Ellinger and Matsuoka, Z. physiol. Chem., **109**, 259 (1920).
[469] Kotake and collaborators, ibid., **195**, 139 (1931); **214**, 1 (1933).

These reactions take place only with $l(+)$-tryptophan, not with the unnatural $d(-)$-variety.[470]

Kynurenine is stable to acids but is unstable in alkaline solution, yielding ammonia, carbon dioxide, o-aminoacetophenone, and kynurenic acid, apparently by way of o-aminobenzoylpyruvic acid.

$$NH_2-C_6H_4-\underset{\overset{|}{CO_2H}}{C}=CH-CH(NH_2)-CO_2H \rightarrow NH_2-C_6H_4-CO-CH_2-CO-CO_2H$$

$$NH_2-C_6H_4-CO-CH_3$$

Kynurenine is also converted into kynurenic acid on administration to dogs, the reaction taking place in the liver.

Similar conversions are brought about by certain microörganisms; *B. subtilis*, for instance, forms kynurenine, kynurenic acid, and anthranilic acid from tryptophan. Of other organisms, some (*B. coli*) break down *l*-tryptophan into indole in the presence, and into indole-3-propionic acid in the absence, of air;[471] some (*B. aminophilus intestinalis*) effect decarboxylation to β-indoleethylamine;[472] some (*Proteus* and *Oidium lactis*, respectively) bring about deamination to varieties of indolelactic acid having opposite configurations;[473] some (yeast) cause both deamination and decarboxylation to tryptophol (β-indoleethanol).[474]

In intestinal putrefaction, tryptophan is broken down into indole and β-methylindole (skatole). These products partly diffuse into the blood stream, where they undergo further chemical alteration. Little is known about the fate of skatole; it is possibly oxidized at the side chain. Indole is oxidized to indoxyl, which is eliminated by the kidney as a sulfuric ester, indican.

$$\underset{NH\!-\!\!-\!\!-CH}{C_6H_4\!-\!\!-COSO_3^-}$$

The presence in the urine of abnormally large amounts of indoxylsulfuric acid, diagnostic of chronic constipation or intestinal obstruction, is indicated by the production of indigo on treatment with oxidizing

[470] Correll, Berg, and Cowan, *J. Biol. Chem.*, **123**, 151 (1938).

[471] Woods, *Biochem. J.*, **29**, 640, 649 (1935).

[472] Berthelot and Bertrand, *Compt. rend.*, **154**, 1826 (1912).

[473] Sasaki and Otsuka, *Biochem. Z.*, **121**, 167 (1921).

[474] Ehrlich, *Ber.*, **45**, 883 (1912).

agents.[475] Indican can be estimated colorimetrically by treatment with triketohydrindene,[476] which yields a red condensation product.

$$C_6H_4 \underset{NH}{\overset{CO}{\diagdown}} C{=}C \underset{CO}{\overset{CO}{\diagdown}} C_6H_4$$

In living mammals, an appreciable part of the tryptophan taken in the normal diet is utilized for protein synthesis. Normal growth is impossible on diets deficient in this essential amino acid, but can be induced by the addition, to such diets, of not only *l*-tryptophan, but *d*-tryptophan or acetyl-*l*-tryptophan. The effect of a large number of derivatives of tryptophan on growth and kynurenic acid production is summarized in the accompanying table. From the results it appears that the biochemi-

	GROWTH STIMULATION	KYNURENIC ACID FORMATION	LITERATURE REFERENCES
l-Tryptophan..	+	+	
d-Tryptophan.....	+	–	477, 478
Acetyl-*l*-tryptophan.......	+	±	477, 478
Acetyl-*d*-tryptophan	–	–	477, 478
Propionyl-*l*-tryptophan............	+	..	479
Benzoyl-*l*-tryptophan.............	–	..	480
Phenacetyl-*l*-tryptophan...........	–	..	479
l-Tryptophan esters......	+	..	479, 480
l-Tryptophan amides..	+	+	481
(+)-Indolelactic acid	+	+	469, 482
(–)-Indolelactic acid.......	–	–	469, 482
Indolepyruvic acid...............	+	+	468, 469
Indolepyruvic acid oxime..	–	–	482
Indolepropionic acid.............	–	..	483
Indoleacrylic acid	–	–	482, 484
Kynurenine..	–	+	469, 485

cal mechanisms respectively involved are different and independent. The behavior of the two forms of acetyltryptophan indicates that the body can hydrolyze the *l* variety specifically, but apparently at so slow a

[475] Salkowski, *Z. physiol. Chem.*, **42**, 213 (1904).
[476] Kumon, *ibid.*, **231**, 205 (1935).
[477] du Vigneaud, Sealock, and Van Etten, *J. Biol. Chem.*, **98**, 565 (1932).
[478] Berg, *ibid.*, **104**, 373 (1934).
[479] Berg and Hanson, *Proc. Iowa Acad. Sci.*, **41**, 165 (1934) [*C. A.*, **29**, 4049 (1935)].
[480] Berg, *J. Biol. Chem.*, **91**, 513 (1931).
[481] Bauguess and Berg, *ibid.*, **106**, 615 (1934).
[482] Bauguess and Berg, *ibid.*, **104**, 675, 691 (1934).
[483] Jackson, *ibid.*, **84**, 1 (1929).
[484] Bauguess and Berg, *Proc. Iowa Acad. Sci.*, **40**, 110 (1933) [*C. A.*, **29**, 2579 (1935)].
[485] Jackson and Jackson, *J. Biol. Chem.*, **96**, 697 (1932).

rate that very little of the resulting tryptophan is diverted from its major function—that of growth stimulation—to the production of kynurenic acid. On the other hand, hydrolysis of the amides (—NH$_2$, —NHEt, —NEt$_2$, —NHC$_6$H$_5$, —NEtC$_6$H$_5$) of l-tryptophan seems to be more rapidly accomplished, as all stimulate growth and produce kynurenic acid more freely than acetyl-l-tryptophan, though not so readily as l-tryptophan. The fact that indolepyruvic acid can also without undue difficulty fulfill both functions of tryptophan, whereas d-tryptophan yields little or no kynurenic acid, may likewise be regarded as indicating a relatively slow conversion of d-tryptophan into l-tryptophan through the pyruvic acid. However, interpretation of the data is complicated by the circumstance that the growth studies were carried out with diets devoid of tryptophan, which was not true of the experiments on the production of kynurenic acid. In the absence of an urgent demand for tryptophan it is conceivable that a related compound such as indolepyruvic acid may be metabolized more extensively by oxidative degradation than by amination to l-tryptophan.

The behavior of the two varieties of indolelactic acid is of interest: the body apparently possesses an enzyme capable of converting the dextrorotatory, but not the levorotatory, variety into the keto acid, and it has been suggested [469] that the dextrorotatory form has the same configura'·... as l-tryptophan, from which it is produced by *Proteus* but not by *Oidium lactis*. Finally, it is clear that, while kynurenine is an intermediate in the production of kynurenic acid, it cannot be biochemically converted into tryptophan.

A study of three methyl derivatives of dl-tryptophan,
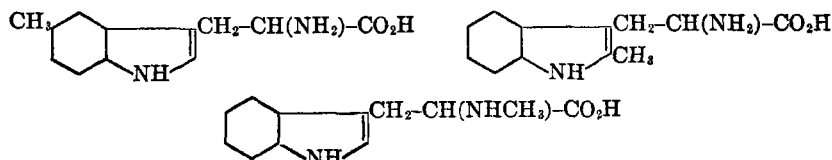
has shown [486] that the power to stimulate growth is not impaired by methylation of the α-nitrogen atom of tryptophan, but is completely inhibited by introduction of a methyl group into either nucleus of the indole structure.

A methyltryptophan in which the pyrrole-nitrogen atom is methylated has been synthesized [487] from indolealdehyde by methylation followed by the Erlenmeyer procedure, sodium lead alloy being employed for reduction of the azlactone.

[486] Gordon and Jackson, *ibid.*, **110**, 151 (1935).
[487] Wieland, Konz, and Mittasch, *Ann.*, **513**, 1 (1934).

The structures shown (reaction scheme with CHO and indole/quinoline-type rings):

CHO → CHO → CH=C-N=CC$_6$H$_5$ →

and product:

CH$_2$—CH-NH$_2$ / CO$_2$H on C$_6$H$_4$ ring with CH$_3$
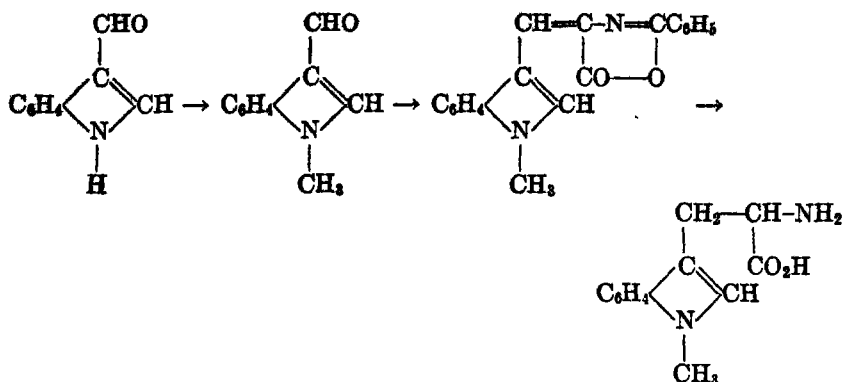
The product is precipitable from acid solution by mercuric sulfate, but fails to give the characteristic color tests with glyoxylic acid and with p-dimethylaminobenzaldehyde.

The trimethylbetaine of l-tryptophan, hypaphorine, has been isolated from the seeds of a Javanese tree.[488] A closely related compound, bufotenine,
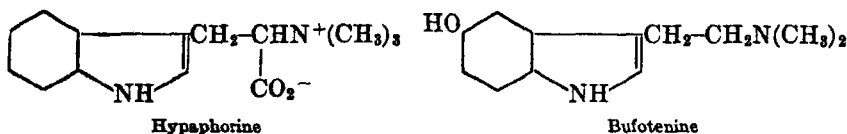
CH$_2$-CHN$^+$(CH$_3$)$_3$
CO$_2$$^-$

Hypaphorine

HO ... CH$_2$-CH$_2$N(CH$_3$)$_2$

Bufotenine

is present in the venomous secretion of the European toad.[487]

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[488] v. Romburgh and Barger, *J. Chem. Soc.*, 99, 2068 (1911).

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CHAPTER 15

ALKALOIDS

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CONTENTS

INTRODUCTION

The term alkaloid, meaning alkali-like, is applied to naturally occurring basic nitrogen compounds, and is, in general usage, limited to those of plant origin. Most of the alkaloids have the nitrogen atom linked in a cyclic structure, are optically active, and show marked physiological activity, although a few substances classified as alkaloids are exceptional in respect to one or more of these characteristics. A variety of open-chain simple bases, as the cholines, amino acids, and phenylalkyl-amines, are distinguished from the true alkaloids, by some authorities, under the name vegetable bases. The distinction is somewhat arbitrary, and ephedrine, mescaline, and a few similar bases will be treated here as alkaloids.

The nomenclature of the individual alkaloids has not been systematized, for historical reasons and because of the complexity of the structures involved. A great many important alkaloids have received names derived from those of plants, as papaverine, hydrastine, berberine. A few are named from their physiological action, as morphine, narcotine, and emetine; several from their physical characteristics, as hygrine and porphyroxine; only one, pelletierine, has been named for an alkaloid chemist. The name of the principal alkaloid with a prefix or suffix is

often applied to the minor alkaloids found in the same plant, as for example in the cinchona series; related bases are sometimes named by transpositions, as narcotine, cotarnine, and tarconine. It is customary to designate isomeric new bases (often transformation products of the natural alkaloid) with such prefixes as *iso*, *pseudo*, *allopseudo*, *neo*, *epi*, or with Greek letters (see the codeine isomers and the methylmorphimethines, p. 1222), or occasionally with a suffixed Roman letter.

The alkaloids as a class are well-crystallized colorless compounds; a few, notably arecoline, sparteine, the hygrines, and most members of the coniine and nicotine groups, are liquids. The liquid alkaloids are generally oxygen-free. Colored alkaloids are rare; berberine is yellow, and the salts of sanguinarine are copper-red. Nearly all alkaloids form crystalline salts, which are often utilized in isolating or purifying the base; the acids usually employed are sulfuric, oxalic, perchloric, tartaric, salicylic, and the halogen acids. Most alkaloids react with alkyl halides, especially methyl iodide, to give crystalline addition products. Secondary amines give with methyl iodide the N-methylated hydriodide, tertiary amines give methiodides, which are of importance for degradative reactions. The so-called alkaloid reagents are used for the detection and often for the identification of minute amounts of the natural bases or their derivatives, and can be divided roughly into precipitants and color reagents. The precipitating reagents combine with alkaloids to give almost insoluble addition products, and thus may serve to demonstrate the presence of alkaloidal material, even in very small quantities, in drugs or plant extracts. A few of the reagents, however, are known to form precipitates with non-alkaloidal classes (proteins and glucosides). The alkaloid chemist utilizes the precipitants as convenient reagents for the approximate estimation of the amount of alkaloid remaining in aqueous solution after filtration or extraction. The precipitates often have a definite, constant composition, and can be employed for analysis; they sometimes crystallize in characteristic forms on the microscope slide, and permit preliminary identification of the alkaloid. Among the more important precipitating reagents may be mentioned Mayer's (potassium mercuric iodide), Sonnenschein's (phosphomolybdic acid), Knorr's (picrolonic acid), Hager's (picric acid), Wagner's (potassium triiodide), Dragendorff's (potassium bismuth iodide), Scheibler's (phosphotungstic acid), and Bertrand's (silicotungstic acid); further, chloroplatinic and chlorauric acids, which are adapted to analytical use. In individual cases the precipitating reagents, especially picric acid, have been used to separate mixtures of alkaloids.

The color reagents mostly consist of dehydrating or oxidizing agents, or combinations of these, to which aldehydes may also be added. The

alkaloidal residue obtained by evaporation of solutions in a porcelain dish is moistened with the reagent, and often warmed, and the color produced is compared with that from known samples. In certain cases, as for example, Lautenschläger's diazosulfanilic acid reagent for morphine, dilute solutions are employed, and the amount of alkaloid present can be determined with the colorimeter. The common color reagents are concentrated sulfuric acid solutions of such substances as formaldehyde (Marquis' reagent), nitric acid (Erdmann's), potassium dichromate (Luchini's), potassium permanganate (Wenzell's), or molybdic acid (Fröhde's).

Alkaloids are found almost exclusively in phanerogams, the seed-bearing plants, for the most part in dicotyledons, seldom in monocotyledons; occurrence in cryptogams is rare (ergot). The same species or genus may contain many different alkaloids, which are, however, usually related in structure. From the opium poppy, for example, ten members of the benzylisoquinoline group have been isolated, which differ chiefly in the nature of peripheral groups, or in the degree of hydrogenation of the nucleus. The four morphine-type alkaloids found in the same plant differ from each other in the same way, and in theory, at least, can be related to the benzylisoquinoline group by the establishment of a single new carbon-to-carbon linkage.[1,2,3] It is indeed difficult to find any case where unrelated alkaloids occur in a single species. A given alkaloid is seldom present in different plant families; berberine, protopine, and the xanthine derivatives are exceptional in this respect. The alkaloidal content may be greatly influenced by selection and cultivation; planters have been especially successful in increasing the quinine yield from the cinchona tree in Java. The function of alkaloids in the plant is still a subject of speculation. The alkaloids are generally concentrated in the living tissue at points of intense cell activity, whence they are often cast aside and stored in such dead structures as the seed hulls or outer bark. They are regarded as by-products of plant metabolism (Tschirch), in contrast to the simple bases and betaines that probably constitute the building units for the formation of plant proteins. Other theories that have been advanced conceive alkaloids to be reserve materials stored for protein synthesis; protective substances discouraging animal or insect attacks; plant stimulants or regulators similar to hormones; or detoxication products, rendered harmless to the plant by methylation, condensation, ring closure, and other synthetic processes.

Alkaloids occur usually in the form of salts of the common natural

[1] Gulland and Robinson, Mem. Proc. Manchester Lit. Phil. Soc., 69, 79 (1925).

[2] Schöpf, Ann., 452, 211 (1927).

[3] Awe, Arch. Pharm., 272, 466 (1934).

organic or inorganic acids, or of acids peculiar to the plant family, as meconic acid in the poppy, or quinic acid in cinchona. Occasionally the alkaloids are present in the free state, because of extremely weak basic properties, e.g., narceine and narcotine. More rarely they exist in combination with sugars, for example the glucoalkaloid solanine (p. 1467), or in the form of amides (piperine), or esters (atropine, cocaine) of organic acids. The crude alkaloid is separated from the powdered plant parts by extraction with water, alcohol, or dilute acids (hydrochloric, sulfuric, or acetic); or the vegetable material may be treated with alkali and the alkaloid extracted by organic solvents. For volatile alkaloids (nicotine and coniine groups), steam distillation is employed. The crude mixture of alkaloids obtained by these methods always contains coloring matter and resins, and is generally purified by repeated crystallization of sparingly soluble salts. Adsorbing agents (charcoals) are frequently used to remove color; occasionally the colored impurities can be destroyed by oxidation, as is the practice in cocaine manufacture.[4] The individual alkaloids are usually separated through differences in solubility of their various salts. The separation may sometimes be accomplished by utilizing differences in basicity of the alkaloids, i.e., fractional extraction or precipitation. In this method, which was developed by Gadamer, a solution of the mixed alkaloids in dilute acid is treated with successive small portions of ammonia or sodium hydroxide, and the liberated alkaloid is extracted with an organic solvent after each addition of alkali. The first fractions will contain the weakly basic alkaloids, the last fractions the more strongly basic. Conversely, a solution of the mixed alkaloids in benzene, ether, or chloroform may be extracted with many small portions of dilute acid, the strongest bases being extracted first.

The first step in structure determination consists in isolating the nitrogen-containing portion of the alkaloid, whether by simple liberation from salts, or by hydrolytic processes as exemplified by the glucoalkaloids and cocaine types. Hydrolysis of new alkaloids must, however, be employed with caution, since in some types of alkaloid structure, for example narcotine, hydrastine, thebaine, strychnine, the basic portion itself may be split or undergo racemization. After determining the empirical formula and the optical rotatory power of the pure alkaloid, the chemist proceeds to ascertain the function of oxygen and nitrogen, how the molecule may be broken into simple fragments, and what the fundamental ring system may be. The presence of oxygen as a phenolic hydroxyl is shown by alkali solubility, ferric chloride reaction, acylation, and alkylation; in the form of an alcoholic hydroxyl by reaction with

[4] Schwyzer, "Die Fabrikation der Alkaloide," Springer, Berlin (1927); "Die Fabrikation pharmazeutischer und chemisch-technischer Produkte," Springer, Berlin (1931).

phosphorus chlorides or thionyl chloride, by acetylation, or occasionally by dehydration or oxidation. Carboxyl groups (arecaidine, narceine) confer solubility in sodium carbonate or ammonia, and their presence may be demonstrated by esterification. Ether-linked methoxyl groups and acetal-linked methylenedioxyl groups occur frequently. Methoxyl groups can be estimated quantitatively by the method of Zeisel[5, 6] or of Vieböck,[7] which involves boiling the substance with concentrated hydriodic acid and determining the amount of methyl iodide formed. The detection and quantitative estimation of the methylenedioxyl group are accomplished by reactions in which formaldehyde is split out by means of sulfuric acid.[6] No other alkoxyl groups have ever been found, a fact that indicates the importance of formaldehyde in the phytochemical synthesis of alkaloids. Many alkaloid structures are so stable that methoxyl or methylenedioxyl groups may be split without other structural changes, whereby the corresponding hydroxy bases are obtained. For this purpose, constant-boiling hydrobromic acid, or aluminum bromide, has proved particularly useful.[5, 9] Carbonyl groups (cryptopine, narceine) may be identified by the usual methods; lactone (narcotine, hydrastine), lactam, or betaine (arecoline, hypaphorine) groups are usually detected by hydrolysis.

The determination of methyl groups on nitrogen is carried out by the method of Herzig and Meyer,[6, 10] which consists in heating the alkaloid hydriodide at 200-300° and estimating the methyl iodide formed. This process may be carried out in combination with the Zeisel analysis for methoxyl groups. Occasionally the methyl group on nitrogen (higher N-alkyl groups are never found *) can be replaced by hydrogen through the action of cyanogen bromide, nitrous acid, alkaline permanganate, or other reagents, yielding secondary amines. These are distinguished by *nor* prefixed to the alkaloid name, but the same prefix is sometimes used to designate bases obtained by demethylation at oxygen.

With few exceptions the nitrogen in alkaloids is in a ring structure, and can be only secondary or tertiary. It is often difficult to distinguish between these two forms. Tests for the secondary amino group which depend upon reactions of active hydrogen cannot be evaluated until the number of other active hydrogens in the molecule is known. Nitrogen

[5] Zeisel, *Monatsh.*, **6**, 989 (1885); **7**, 406 (1886).

[6] Meyer, "Analyse und Konstitutionsermittlung organischer Verbindungen," Springer, Berlin (1922).

[7] Vieböck, *Ber.*, **63**, 2818, 3207 (1930).

[8] Schöpf and Thierfelder, *Ann.*, **497**, 22 (1932).

[9] Mosettig and Burger, *J. Am. Chem. Soc.*, **52**, 2988 (1930).

[10] Herzig and Meyer, *Monatsh.*, **15**, 613 (1894); **16**, 599 (1895); **18**, 379 (1897).

* This statement must now be qualified, since Freudenberg [*Ber.*, **69**, 1962 (1936)] has proved that aconitine contains an N-ethyl group.

is generally assumed to be tertiary if the usual reactions [6, 11, 12] for secondary nitrogen are negative.[*] The ability to form amine oxides with 30 per cent hydrogen peroxide, and to react with 1,5-dibromopentane [13] with formation of pentane diammonium bromides, is sometimes used to characterize tertiary bases. The most generally applicable method for ascertaining structure is exhaustive methylation, also known as the Hofmann degradation. This depends upon the tendency of many quaternary ammonium hydroxides to decompose with loss of water and scission of a carbon-to-nitrogen linkage when heated,[†] and often gives immediate structural information. With an open-chain tertiary amine, a single methylation and decomposition suffices to eliminate nitrogen as trimethylamine.

$$RCH_2CH_2N(CH_3)_2 \rightarrow RCH_2CH_2N(CH_3)_3I \rightarrow RCH_2CH_2N(CH_3)_3OH$$
$$\rightarrow RCH{=}CH_2 + N(CH_3)_3 + H_2O$$

If, on the other hand, two of the nitrogen valences are involved in a hydrogenated ring structure, the first decomposition yields an unsaturated open-chain amine, with which the process must be repeated before nitrogen can be split out and the carbon skeleton exposed.

[11] Houben, "Die Methoden der organ. Chemie," 2nd ed., Thieme, Leipzig (1925), Vol. IV, pp. 369, 502.

[12] Rosenthaler, "Der Nachweis organ. Verbindungen," 2nd ed., Enke, Stuttgart (1923), p. 514.

[13] von Braun, *Ber.*, **41**, 2156 (1908).

[*] Such reactions must be interpreted with caution. Some tertiary bases (e.g., apomorphine, morphothebaine) yield N-benzoyl derivatives through ring scission.

[†] For a discussion of the probable mechanism see Schlenk-Bergmann "Ausführliches Lehrbuch der organischen Chemie," Deuticke, Leipzig and Vienna (1932), Vol. I, p. 55.

See for example the degradation of pseudopelletierine to cyclöoctadiene, p. 1183. In accordance with a suggestion of Willstätter,[14] the unsaturated amine formed in the first step of exhaustive methylation usually receives the prefix des-. The suffix -methine is also sometimes used to designate these compounds (see the codeine series). Where nitrogen is linked in ring structures through three valences, three methylations and decompositions are necessary to eliminate the nitrogen. This is true of canadine or tetrahydroberberine,[15] in which the following ring system is present:

The Hofmann degradation is not applicable to all types of alkaloids; it fails with unhydrogenated pyridine, quinoline, and isoquinoline derivatives, and with hydrogenated quinolines. A useful modification, the Emde degradation, involves treating an alcoholic or aqueous solution of the quaternary halide with sodium amalgam. The Emde degradation may yield the same degradation product as the Hofmann method, or a reduced derivative, sometimes a mixture of the two. It often succeeds with ring systems that cannot be degraded according to Hofmann. Tetrahydrodimethylquinolinium halides, for example, split off methyl alcohol to give N-methyltetrahydroquinoline when heated with alkali. By Emde's process, the principal product is γ-dimethylaminopropylbenzene.

[14] Willstätter, *Ann.,* **317,** 268 (1901).
[15] McDavid, Perkin, and Robinson, *J. Chem. Soc.,* **101,** 1218 (1912).

With tetrahydrodimethylisoquinolinium halides, both methods yield the same primary product, (o-vinylbenzyl)dimethylamine, but further degradation by Hofmann is unsuccessful, whereas application of the Emde method results in o-methylstyrene.[16, 17]

has been discovered recently [18] that some quaternary ammonium halides can be degraded smoothly by catalytic hydrogenation, but the method has been applied to only a few alkaloids.[19]

Two other methods for opening nitrogen-containing rings were devised by J. von Braun.[20] One of these, applicable to cyclic secondary amines, consists in treating the benzoyl derivative of the amine with phosphorus halides. From benzoylpiperidine, for example, ε-chloroamylamine is obtained.

When the reaction is carried out at a higher temperature, 1,5-dichloropentane and benzonitrile are the products. The second von Braun degradation makes use of cyanogen bromide, CNBr. This reagent reacts with tertiary nitrogen compounds to break one carbon-to-nitrogen linkage, the cyano group becoming attached to nitrogen and the bromine atom to carbon. Cyclic N-alkyl compounds may be dealkylated with formation of a cyclic N-cyano derivative, or the ring may be opened with formation

[16] Emde and Kull, Arch. Pharm., 272, 469 (1934).
[17] Emde, Ann., 391, 88 (1912).
[18] Emde and Kull, Arch. Pharm., 274, 173 (1936).
[19] Achmatowicz and Bochwic, C. A., 29, 6240 (1935).
[20] von Braun, Ber., 37, 2915, 4723 (1904); 40, 3914 (1907).

of a cyanamino derivative, depending upon the structural features adjacent to the nitrogen atom. The cyanogen bromide degradation is of interest because it often succeeds with compounds that resist the Hofmann degradation, further, because it opens the ring at a different point from the latter. The degradation of hydrocotarnine (p. 1220), for example, proceeds thus: [21]

Other vigorous degradations are often employed to determine the fundamental structures present. Oxidative methods have been widely used; see, for example, nicotine, cinchonine, papaverine. Oxidizing agents employed are chosen according to the degree of degradation desired and the stability of the structures present. Mild oxidizing agents, as silver acetate, mercurous acetate, or alkaline potassium ferricyanide, may cause only partial dehydrogenation. Chromic acid and alkaline permanganate have been used most frequently, and by control of temperature and concentration it is often possible to oxidize in steps until only the most resistant nuclei remain unattacked. Lead peroxide or manganese dioxide in sulfuric acid, nitric acid, hydrogen peroxide, and alkaline solutions of bromine or iodine have been used in individual cases. Distillation over hot zinc dust breaks the molecule down to stable ring systems; morphine gives phenanthrene, cinchonine yields quinoline and picoline, strychnine gives lutidine and carbazole. Other reductive methods, especially sodium and alcohol, and catalytic hydrogenation, have helped to establish relationships between alkaloids that differ in degree of oxidation, or owe their isomerism to differences in the position of unsaturated linkages. Fusion with alkali, heating with bromine or phosphorus halides, and similar drastic reactions are often used in degrading the alkaloids to known substances. From the fragments thus obtained it is sometimes possible to make a reasonable structural picture of the alkaloid itself.

The constitution of a considerable number of alkaloids is now known with certainty, and for many the structural formula has been confirmed

[21] von Braun, *Ber.*, **49**, 2624 (1916).

by synthesis. Especially noteworthy are the methods developed in recent years by C. Schöpf and G. Hahn for the synthesis of alkaloids under conditions comparable to those existing in the living plant. These investigations, which bring welcome support to the inspiring speculations of Winterstein and Trier, and Robert Robinson, are summarized in the concluding paragraphs of this chapter. The discovery of new and often medicinally important alkaloids is proceeding more rapidly than structure elucidation, and there still remain whole groups of long-known valuable alkaloids concerning whose structure there is little knowledge (for example, the aconite and veratrine groups), as well as many individual alkaloids whose empirical formula is still uncertain. There are few fields of organic chemistry where so many unsolved problems lie at hand. It is possible to discuss here only a limited number of the more important alkaloids, which have been chosen as representatives of various heterocyclic systems most frequently found in nature. For more complete information, the numerous exhaustive textbooks on alkaloids must be consulted.[22]

PHENYLALKYLAMINE GROUP

The phenylalkylamine bases ephedrine and hordenine depart from the conventional alkaloid definition in possessing an open-chain amine structure. Ephedrine has in other respects typical alkaloid properties, and especially because of its physiological action deserves discussion among the alkaloids. Hordenine, because of its obvious relationship to the cyclic bases from *Anhalonium* species, is treated under the mescal alkaloids (p. 1209).

Ephedra Bases. The Chinese herb known as Ma huang, which has been used in the alleviation of a variety of ailments for some 5000 years, consists principally of the dried parts of *Ephedra sinica* or *E. equisetina*. At least six bases are present, of which the most important is *l*-ephedrine. It is accompanied by *d*-pseudoephedrine, *l*-methylephedrine, *d*-methylpseudoephedrine, *l*-norephedrine, and *d*-norpseudoephedrine. All these constituents, as well as their optical antipodes and racemates, have been synthesized.

$$C_6H_5\overset{\displaystyle \overset{H}{|}}{C}\overset{\displaystyle \overset{H}{|}}{-}\overset{\displaystyle |}{C}-CH_3$$
$$\underset{\displaystyle OH}{|}\quad \underset{\displaystyle NHCH_3}{|}$$

I. Ephedrine

$$C_6H_5\overset{\displaystyle \overset{OH}{|}}{C}\overset{\displaystyle \overset{H}{|}}{-}\overset{\displaystyle |}{C}-CH_3$$
$$\underset{\displaystyle H}{|}\quad \underset{\displaystyle NHCH_3}{|}$$

II. Pseudoephedrine

[22] Henry, "The Plant Alkaloids," Blakiston, Philadelphia (1939); Schmidt-Grafe, "Alkaloide," Urban and Schwarzenberg, Berlin (1920); Seka, "Alkaloide," Urban and Schwarzenberg, Berlin (1927, 1933). Other textbooks and review articles are listed under "General References" on p. 1257.

Ephedrine contains two dissimilar asymmetric carbon atoms, so that four optically active isomers (p. 229) are possible. Of these, the natural bases *l*-ephedrine and *d*-pseudoephedrine are diastereoisomers, and are mutually interconvertible. Their optical opposites are known only by synthesis. The first structural determination in the series was carried out by Ladenburg,[23] who succeeded in demonstrating the nature of *d*-pseudoephedrine. It is a secondary base containing an alcoholic hydroxyl group, as indicated by the formation of a nitroso derivative and a dibenzoyl compound. The presence of a methyl group on nitrogen is evident from the appearance of methylamine when the base is degraded with hydrochloric acid, and the simultaneous formation of methylamine homologs shows that the methylamino group is not located at the end of a chain. Oxidation of pseudoephedrine gives benzoic acid or benzaldehyde, pointing to a hydroxyl on the carbon adjacent to the benzene ring. Evidence from these degradations, therefore, shows the probable structure for ephedrine and pseudoephedrine to be that of a propylbenzene carrying a hydroxyl and a methylamino group in the 1- and 2-positions of the side chain.

The formulas derived for the ephedra bases from degradative reactions have been confirmed by numerous syntheses.[24] Späth and Göhring prepared all the ephedrine isomers from 1-phenyl-1-methoxy-2-bromopropane. This was converted with methylamine to the corresponding 2-methylaminopropane, which, on treatment with hydrobromic acid, yielded 1-phenyl-1-hydroxy-2-methylaminopropane, racemic pseudoephedrine. The racemic base was resolved into the known (natural) *d*-pseudoephedrine and its enantiomorph *l*-pseudoephedrine, or isomerized to *d,l*-ephedrine, which could likewise be resolved into the enantiomorphic *d*- and *l*- (natural) ephedrines.

$$\underset{\underset{\text{Br}}{|}}{\overset{\overset{\text{OCH}_3}{|}}{\text{C}_6\text{H}_5\text{CH—CH—CH}_3}} \rightarrow \underset{\underset{\text{NHCH}_3}{|}}{\overset{\overset{\text{OCH}_3}{|}}{\text{C}_6\text{H}_5\text{CH—CH—CH}_3}} \rightarrow \underset{\underset{\text{NHCH}_3}{|}}{\overset{\overset{\text{OH}}{|}}{\text{C}_6\text{H}_5\text{CH—CH—CH}_3}}$$

d,l-Pseudoephedrine

The action of acids converts *l*-ephedrine to *d*-pseudoephedrine, and prolonged heating with hydrochloric acid reverses this change. The rearrangement apparently takes place through replacement of the hydroxyl group by halogen, followed by hydrolysis of the 1-phenyl-1-halogeno-2-

[23] Ladenburg and Oelschlägel, *Ber.*, **22**, 1823 (1889).

[24] Eberhard, *Arch. Pharm.*, **253**, 62 (1915); **258**, 97 (1920); Späth and Göhring, *Monatsh.*, **41**, 319 (1920); (*d*) Nagai and Kanao, *Ann.*, **470**, 157 (1929); Manske and Johnson, *J. Am. Chem. Soc.*, **51**, 580 (1929); Skita and Keil, *Ber.*, **62**, 1142 (1929).

methylaminopropane with inversion at the number one carbon atom.[25] The configuration of *l*-ephedrine and of *d*-pseudoephedrine as in formulas I and II was established by Freudenberg through relationships to synthetic material of known configuration.[26] The synthesis of the natural ephedrine homologs is described [24*d*] by Nagai and Kanao.

The *l*-ephedrine of commerce is mostly of synthetic origin. The laborious and wasteful separation of *d,l*-isomers is avoided by an ingenious use of enzymes. Sugar is fermented in the presence of benzaldehyde, whereby *levo*-1-phenyl-2-ketopropanol-1

$$C_6H_5-\underset{\underset{OH}{|}}{CH}-\underset{\underset{O}{||}}{C}-CH_3$$

is formed, probably through an enzymatic condensation between benzaldehyde and acetaldehyde. The *levo* ketone is then condensed with methylamine under reducing conditions, giving *l*-ephedrine directly.[27]

Ephedrine has a strong mydriatic action. It contracts the blood vessels and causes a prolonged rise in blood pressure. Its astringent action on mucous membrane is utilized in treating allergic conditions such as hay-fever and asthma, and in shrinking engorged nasal tissues.

PYRIDINE GROUP—HEMLOCK, PEPPER, POMEGRANATE, ARECA NUT, AND CASTOR-BEAN ALKALOIDS

Hemlock Alkaloids. The hemlock herb or spotted cowbane, *Conium maculatum*, contains five alkaloids, coniine $C_8H_{17}N$, γ-coniceine $C_8H_{15}N$, conhydrine $C_8H_{17}ON$, pseudoconhydrine $C_8H_{17}ON$, and N-methylconiine $C_9H_{19}N$, in combination with malic and caffeic acids. Coniine was isolated in 1831, but its constitution was not determined until about fifty years later (Hofmann).[28] Coniine is a strongly alkaline, dextrorotatory liquid, of penetrating odor and burning taste. When its hydrochloride is distilled with zinc dust, a new base, conyrine, containing six less hydrogen atoms, is formed. Conyrine can be reduced again to (optically inactive) coniine with concentrated hydriodic acid.

$$C_8H_{17}N \rightarrow C_8H_{11}N + 6H$$
Coniine   Conyrine

Further information on the structure of coniine was obtained by the oxidation of conyrine, which yielded α-picolinic acid.

[25] Emde, *Helv. Chim. Acta*, **12**, 365 (1929); Emde and Spaenhauer, *ibid.*, **13**, 3 (1930).

[26] Freudenberg, Schoeffel, and Braun, *J. Am. Chem. Soc.*, **54**, 234 (1932); Freudenberg and Nikolai, *Ann.*, **510**, 223 (1934).

[27] Hildebrandt and Klavehn, U. S. pat. 1,956,950.

[28] Hofmann, *Ber.*, **18**, 5, 109 (1885).

I. Coniine   II. Conyrine   III. α-Picolinic Acid

Since two carbon atoms were lost in the oxidation, conyrine was thus shown to be an α-propylpyridine. It was found not identical with the known α-isopropylpyridine and was therefore assigned the alternative formula, α-*n*-propylpyridine. Coniine is the dextro form of α-*n*-propylpiperidine.

Formula I is in accord with the behavior of coniine as a secondary amine in its reactions with acylating agents and with nitrous acid, and found confirmation in Ladenburg's synthesis [29] in 1886, the first synthesis of an alkaloid. In general, when pyridinium alkyl halides are heated under pressure, the alkyl group shifts to the α- or γ-position, yielding the corresponding alkylpyridines. Ladenburg's attempts to prepare conyrine by this method failed because of isomerization of the *n*-propyl group to isopropyl under the drastic conditions involved. The coniine synthesis was finally accomplished by condensation of α-picoline with paraldehyde and reduction of the condensation product to α-propylpiperidine with sodium and alcohol. The optically inactive product yielded on resolution with tartaric acid a dextrorotatory base identical with coniine (see also Hess).[30]

γ-Coniceine contains two hydrogen atoms less than coniine. It is optically inactive, hence the asymmetric carbon atom of coniine must be involved in the unsaturation; on reduction, γ-coniceine gives d,l-coniine. γ-Coniceine can be prepared from chloro- or bromo-coniine by the action of alkali, or from conhydrine by dehydration, facts which find an explanation in formula IV. γ-Coniceine was synthesized by Gabriel.[31]

Conhydrine represents a coniine in which an alcoholic hydroxyl group replaces a hydrogen atom in the side chain. The position of this hydroxyl was long uncertain, but has been proved by the identity of the product of N-methylation and oxidation, methylconhydrinone (VI), with the

IV. γ-Coniceine   V. Conhydrine   VI. Methylconhydrinone

[29] Ladenburg, *Ber.*, **19**, 439, 2578 (1886).
[30] Hess and Weltzien, *Ber.*, **53**, 139 (1920).
[31] Gabriel, *Ber.*, **42**, 4059 (1909).

synthetic 1-(α-N-methylpiperidyl)-1-propanone.[32]   Conhydrine can be reduced to coniine.   On oxidation it yields either the ketone d,l-conhydrinone, or pipecolinic acid; the former is identical with synthetic 1-(α-piperidyl)-1-propanone.

Pseudoconhydrine is a structural isomer of conhydrine, which it resembles closely.   It can be transformed through iodoconiine to d-coniine, or dehydrated to pseudoconiceine, an isomer of the γ-coniceine mentioned above.   Späth [33] showed by degradation that pseudoconhydrine has the hydroxyl group in the piperidine ring, at the 5-position.   The pseudoconhydrinemethine resulting from the first step in the Hofmann degradation was hydrogenated; the dihydro derivative, on oxidation, yielded enanthic acid (n-heptylic acid), or on further degradation gave 1,2-epoxyoctane.   A portion of the dihydro methine, undergoing the normal Hofmann degradation, gave 2-octanone, leaving no doubt as to the position of the hydroxyl in pseudoconhydrine.

N-Methylconiine occurs naturally in both d- and l-forms. d-N-Methylconiine can be prepared from coniine by methylation, the d,l-form from the pomegranate alkaloid methylisopelletierine (p. 1184) by reduction.

The conium alkaloids cause paralysis of the motor nerve endings, and all are poisonous.   The hemlock drink used in ancient times to inflict the death penalty owed its toxicity to these bases.

Pepper Alkaloids.   The alkaloid piperine, $C_{17}H_{19}O_3N$, occurs to the extent of about 5 to 9 per cent in the fruit of *Piper nigrum*, the source of black and of white pepper; it is present in lesser amounts in other *Piper* species.   The sharp taste of pepper is apparently not due to piperine, but rather to an isomer, chavicine.[34]   A third base, piperovatine, is also known.

Piperine is a very weak, optically inactive base, yielding on hydrolysis

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[32] Hess and Grau, *Ann.*, **441**, 101 (1925); Späth and Adler, *Monatsh.*, **63**, 127 (1933).
[33] Späth, *Ber.*, **66**, 591 (1933).
[34] Ott and Lüdemann, *Ber.*, **57**, 214 (1924).

piperic acid and piperidine. The latter substance first became known from this source. Piperic acid is unsaturated, and adds four atoms of bromine or of hydrogen. On oxidation with permanganate, it gives piperonal, and finally piperonylic acid. Piperonylic acid breaks down to protocatechuic acid and carbonaceous products when heated with hydrochloric acid at 170°; conversely, it can be prepared from protocatechuic acid by the action of methylene iodide and alkali. Its constitution as the methylene ether of protocatechuic acid is evident.

HO— HO— COOH
I. Protocatechuic acid

H_2C O— O— COOH
II. Piperonylic acid

Piperic acid differs from piperonylic acid by C_4H_4, which must be located between the aromatic nucleus and the carboxyl group in order to explain the oxidation of piperic acid. The formula thus obtained (III) was confirmed by Ladenburg's synthesis.[35]

H_2C O— O— CH—CH ∥ ∥ CH CHCOOH
III. Piperic acid

H_2C O— O— CH—CH ∥ ∥ CH CH—CO
IV. Piperine

Piperine is the amide of piperic acid with piperidine, and was prepared by Rügheimer[36] from piperoyl chloride and piperidine before either of the components had been synthesized. Except as a local irritant, piperine is practically without physiological action.

Chavicine is the piperidine amide of chavicic acid. The latter is a geometrical isomer (*cis-cis* form) of piperic acid (*trans-trans* form).[37]

Pomegranate Alkaloids. The bark of the pomegranate tree (*Punica granatum*) contains four or more low-melting or liquid alkaloids discovered in 1877 by Tanret, and named pelletierines in honor of the French alkaloid chemist Pelletier. The existence and nomenclature of some of the bases have been the subjects of considerable dispute, and only those of well-established constitution will be discussed here.

The chief alkaloid, pseudopelletierine (N-methylgranatonine), $C_9H_{15}ON$, contains two piperidine rings having nitrogen and two carbon atoms in common, and is closely related to tropinone (p. 1195). The

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[35] Ladenburg and Scholtz, *Ber.*, **27**, 2958 (1894).

[36] Rügheimer, *Ber.*, **15**, 1390 (1882).

[37] Ott and Eichler, *Ber.*, **55**, 2653 (1922).

nitrogen atom is tertiary and carries a methyl group. The single oxygen atom is linked in a ketone group, which stands between two methylene groups, as is shown by the ability of pseudopelletierine to form an oxime, and dibenzylidene or diisonitroso derivatives. By reduction at the carbonyl group, a secondary alcohol, methylgranatoline, is obtained. This base can be converted through a series of intermediates to granatanine, the parent substance of the series. Granatanine is a homolog of norhydrotropidine in the tropine series.[38]

$$
\begin{array}{lll}
CH_2 & CH & CH_2 \\
| & | & | \\
CH_2 & N\text{—}CH_3 & CO \\
| & | & | \\
CH_2 & CH & CH_2
\end{array}
\qquad
\begin{array}{lll}
CH_2 & CH & CH_2 \\
| & | & | \\
CH_2 & NH & CH_2 \\
| & | & | \\
CH_2 & CH & CH_2
\end{array}
$$

I. Pseudopelletierine   II. Granatanine

The constitution of pseudopelletierine rests upon degradations and synthesis. Distillation of granatanine hydrochloride over zinc dust gives α-propylpyridine, a degradation parallel to that of norhydrotropidine to α-ethylpyridine.[39] Pseudopelletierine yields on oxidation a dibasic acid, methylgranatic acid, which still has a piperidine ring intact and contains the same number of carbon atoms as the starting material. Exhaustive methylation of methylgranatic acid leads through IV and V to suberic

$$
\begin{array}{lll}
CH_2 & CH & CH_2 \\
| & | & | \\
CH_2 & NCH_3 & COOCH_3 \\
| & | & \\
CH_2 & CH & COOCH_3
\end{array}
\qquad
\begin{array}{lll}
CH_2 & CH & CH_2 \\
| & | & | \\
CH_2 & N(CH_3)_2 & COOCH_3 \\
| & & \\
CH & CH & COOCH_3
\end{array}
$$

III. Methylgranatic ester   IV. Dimethylgranatenic ester

acid,[40] demonstrating the presence of an unbranched eight-carbon chain in pseudopelletierine.

$$
\begin{array}{l}
CH_2\text{—}CH=CH\text{—}COOH \\
| \\
CH_2 \\
| \\
CH=CH\text{—}COOH
\end{array}
\qquad
\begin{array}{l}
CH_2\text{—}CH_2\text{—}CH_2\text{—}COOH \\
| \\
CH_2 \\
| \\
CH_2\text{—}CH_2\text{—}COOH
\end{array}
$$

V. Homopiperylenedicarboxylic acid   VI Suberic acid

Pseudopelletierine itself was degraded by Willstätter[41] through methylgranatanine to cycloöctadiene, which could be reduced to cycloöctane, or dehydrogenated (by the device described on p. 1196 for the tropilidene

[38] Ciamician and Silber, *Ber.*, **26**, 2738 (1893).
[39] Ciamician and Silber, *Ber.*, **27**, 2850 (1894).
[40] Piccinini, *Gazz. chim. ital.*, [II] **29**, 104 (1899) [*Chem. Zentr.*, II, 808 (1899)].
[41] Willstätter and Waser, *Ber.*, **43**, 1176 (1910); **44**, 3423 (1911).

synthesis) to the cyclooctatetraene so significant to theories concerning aromatic ring structure (p. 129).

$$
\begin{array}{lll}
\text{CH}_2\text{---CH-------CH}_2 & \text{CH}_2\text{---CH-------CH}_2 & \text{CH}\text{==CH---CH}_2 \\
\mid \qquad\qquad\quad \mid & \mid \qquad\qquad\quad \mid & \mid \qquad\qquad \mid \\
\text{CH}_2 \ \ \text{N(CH}_3\text{)}_2\text{OH} \ \ \text{CH}_2 & \text{CH}_2 \ \ \text{N(CH}_3\text{)}_2 \ \ \text{CH}_2 & \text{CH}_2 \qquad\qquad \text{CH}_2 \\
\mid \qquad\qquad\quad \mid & \mid \qquad\qquad\quad \mid & \mid \qquad\qquad \mid \\
\text{CH}_2\text{---CH-------CH}_2 & \text{CH}_2\text{---CH}\text{====CH} & \text{CH}_2\text{---CH==CH}
\end{array}
$$

VII. Methylgranatanine   VIII. des-Dimethyl-   IX. Cyclooctadiene
methohydroxide   granatanine

Pseudopelletierine was synthesized by Menzies and Robinson [42] through a reaction developed as a result of theoretical speculations on the mode of formation of the alkaloid in the plant. Glutaric aldehyde, methylamine, and calcium acetonedicarboxylate were condensed, the product acidified, and the free dibasic acid distilled in a high vacuum, yielding pseudopelletierine.

$$
\begin{array}{l}
\text{CH}_2\text{---CHO} \qquad\qquad\qquad \text{CH}_2\text{---COO} \ \tfrac{\text{Ca}}{2} \\
\mid \\
\text{CH}_2 \qquad + \ \text{CH}_3\text{NH}_2 \ + \ \text{CO} \qquad\qquad \rightarrow \\
\mid \\
\text{CH}_2\text{---CHO} \qquad\qquad\qquad \text{CH}_2\text{---COO} \ \tfrac{\text{Ca}}{2}
\end{array}
$$

$$
\begin{array}{ll}
\text{CH}_2\text{---CH-----CH---COO} \ \tfrac{\text{Ca}}{2} & \text{CH}_2\text{---CH-----CH}_2 \\
\mid \qquad\; \mid \qquad\; \mid & \mid \qquad\; \mid \qquad\; \mid \\
\text{CH}_2 \ \ \text{NCH}_3 \ \ \text{CO} \qquad\qquad \rightarrow & \text{CH}_2 \ \ \text{NCH}_3 \ \ \text{CO} \\
\mid \qquad\; \mid \qquad\; \mid & \mid \qquad\; \mid \qquad\; \mid \\
\text{CH}_2\text{---CH-----CH---COO} \ \tfrac{\text{Ca}}{2} & \text{CH}_2\text{---CH-----CH}_2
\end{array}
$$

The synthesis of pseudopelletierine by a parallel reaction, under simulated physiological conditions, is discussed at the end of this chapter.

Pelletierine (often called punicine), $C_8H_{15}ON$, is an aldehyde possessing the carbon-nitrogen skeleton of coniine, to which it can be reduced. The oxime of pelletierine gives on dehydration a nitrile, which is saponifiable to pelletieric acid, identical with α-piperidylpropionic acid, whence the structure X follows for pelletierine. This alkaloid, in spite of its simple formula, has not been synthesized.*

X. Pelletierine   XI. Pelletieric acid

[42] Menzies and Robinson, J. Chem. Soc., 125, 2163 (1924).

* For a discussion of the difficulties involved, see Spielman, Swadesh, and Mortenson, J. Org. Chem., 6, 780 (1941).

$$H_2C \underset{\underset{H}{N}}{\overset{CH_2}{\underset{(CH_3)}{\bigcirc}}} CH_2COCH_3$$

XII. Isopelletierine (Methylisopelletierine)

Isopelletierine and methylisopelletierine occur in very small amounts in pomegranate bark. The bases react readily with ketone reagents, and the course of oxidation shows that the carbonyl group must be in the side chain. Methylisopelletierine yields on oxidation N-methylpipecolinic acid, on reduction d,l-methylconiine (p. 1180).

$$\begin{array}{ccc} CH_2 & CH & CH_2 \\ | & | & | \\ CH_2 & NCH_3 & CO \\ | & | & | \\ CH_2 & CH_2 & CH_3 \end{array}$$

XIIa

$$H_2C \underset{\underset{CH_3}{N}}{\overset{CH_2}{\bigcirc}} CH_2COOH$$

XIII. N-Methylpipecolinic acid

$$H_2C \underset{\underset{CH_3}{N}}{\overset{CH_2}{\bigcirc}} CH_2CH_2CH_3$$

XIV. Methylconiine

The position of the carbonyl group in the side chain was determined by Hess[32, 43] and Meisenheimer[44] through the identity of methylisopelletierine with 1-(α-N-methylpiperidyl)-2-propanone. A slight rearrangement of the methylisopelletierine formula (XIIa) shows the relationship of the piperidine type pomegranate alkaloids to the condensed ring system of pseudopelletierine.

Pelletierine, usually as a mixture of the pomegranate alkaloids consisting chiefly of pseudopelletierine and isopelletierine, is used as an anthelmintic; it acts specifically on tapeworms.

Areca Nut Alkaloids. The fruit of the betel palm, *Areca catechu*, is used as a mild stimulant and narcotic by some 200,000,000 persons in India, the Philippines, and the islands of the Pacific and Indian Oceans. Betel chewing is one of the most widespread habits of man. The chew usually consists of a piece of areca nut rolled in a leaf of the betel pepper (*Piper betle*) with some lime and a little gambir, tobacco, or catechu. The combination is chewed throughout the day, and often held in the mouth at night. It stimulates excessive salivation, and the saliva is colored blood-red by the action of the lime and gambir on the coloring matter of the areca nuts. The teeth are blackened rapidly. The addict experiences a feeling of well-being, good humor, and contentment. The craving for the drug is intense, but the habit does not appear to cause any degeneration.[45] Of the five alkaloids that have been isolated from

[43] Hess and Littmann, *Ann.*, **494**, 7 (1932).
[44] Meisenheimer and Mahler, *Ann.*, **462**, 301 (1928).
[45] Lewin, "Phantastica. Narcotic and Stimulating Drugs," Dutton, New York (1931).

areca nuts, arecoline is the most important in respect to physiological action, but other substances present in the nuts probably contribute to the intoxicating effect.

Arecoline, $C_9H_{13}O_2N$, is an optically inactive liquid base, present to the extent of about 0.1 per cent in areca nuts. On hydrolysis it is split into methyl alcohol and arecaidine, $C_7H_{11}O_2N$, another alkaloid that is found in smaller amounts in the nuts. Arecaidine is amphoteric; it forms salts both with acids and with alkalies. On esterification with methyl alcohol it is converted to arecoline. The nitrogen atom carries a methyl group that can be split off as methyl chloride by hydrochloric acid at 240°; on treatment with lime, methylamine is formed.

The formula of arecaidine was thus resolved into

$$CH_3N < C_5H_7 - COOH$$

which led Jahns to the conception that it must be a partly saturated pyridine derivative related to nicotinic acid. This theory was confirmed by synthesis; [46] nicotinic acid methochloride, on reduction with tin and hydrochloric acid, yielded arecaidine.

I. Nicotinic acid methochloride   II. Arecaidine

The optical inactivity of arecaidine leaves only the 2,3- and 3,4-positions in question for the double linkage. This uncertainty was eliminated by the synthesis of Wohl. [47] Acrolein was converted by the action of alcohol and hydrogen chloride into β-chloropropionaldehyde acetal, and two molecules of this product were condensed with methylamine. The resulting methylaminodipropionaldehyde diacetal gave on hydrolysis the dialdehyde, which underwent ring closure with loss of water, yielding the aldehyde corresponding to arecaidine. This was transformed through the oxime and nitrile to the acid, arecaidine.

[46] Jahns, *Ber.*, **21**, 3404 (1888); **23**, 2972 (1890); **24**, 2615 (1891); *Arch. Pharm.*, **229**, 669 (1891).

[47] Wohl and Johnson, *Ber.*, **40**, 4712 (1907).

Arecaidine can also be considered the tetrahydro derivative of the betaine type, trigonelline (nicotinic acid methyl betaine). For arecoline, the corresponding quaternary ammonium formula (IIIa) is in better accord with the physiological action than the ester formula (III).

III. Arecoline   IIIa   IV. Guvacine

Guvacine (norarecaidine) and guvacoline (norarecoline) are minor alkaloids, related as acid and methyl ester. The constitution of the pair is evident from the identity of guvacine with 1,2,5,6-tetrahydronicotinic acid, further from the conversion of guvacine into arecaidine by N-methylation.

Arecoline stimulates salivation and perspiration; in larger doses it kills by respiratory paralysis. Areca nut extract, as well as arecoline, has vermifugal action and is used for this effect in veterinary medicine. Betel chewers, nevertheless, are often afflicted with intestinal parasites; the alkaloids probably reach the intestinal tract in too low concentration to be effective.

Castor-Bean Alkaloid. Ricinine, $C_8H_8O_2N_2$, occurs in the seeds and especially in the young plants of *Ricinus communis* (castor-oil plant); it is one of the few alkaloids that is found unaccompanied by others. Ricinine is optically inactive and so weakly basic that it forms no salts.

When ricinine is distilled with zinc dust, pyridine is obtained; catalytic reduction, on the other hand, proceeds with addition of four hydrogen atoms (tetrahydroricinine), facts that point to the presence of a dihydrogenated pyridine nucleus in ricinine. On treatment with alkali, ricinine yields methyl alcohol and the compound $C_7H_6O_2N_2$, which was named ricininic acid (III) in the belief that ricinine was its methyl ester. With fuming hydrochloric acid at 150° ricinine (likewise ricininic acid) gives carbon dioxide, ammonia, and the base $C_6H_7O_2N$, which Späth [48] showed by synthesis to be 4-hydroxy-1-methyl-2-pyridone (II).

I. Ricinine   II. 4-Hydroxy-1-methyl-2-pyridone

[48] Späth and Tschelnitz, *Monatsh.*, **42**, 251 (1921).

The ricinine structural problem was solved by a study of ricinidine (V), a product obtained by chlorination of ricininic acid with phosphorus oxychloride, and reductive elimination of chlorine. It was found that ricinidine, $C_7H_6ON_2$, could be hydrolyzed in two steps. The addition of one molecule of water gave an amide, $C_7H_8O_2N_2$ (VI), which in the second stage of hydrolysis lost ammonia and was transformed to a carboxylic acid (VII).

III. Ricininic acid   IV. Chlororicinidine   V. Ricinidine

VI. 1-Methyl-2-pyridone-3-carboxylic amide   VII. 1-Methyl-2-pyridone-3-carboxylic acid

The structure of the 1-methyl-2-pyridone-3-carboxylic acid so obtained was demonstrated by synthesizing the three possible isomers. The formula thus derived for ricinine was confirmed by Späth's synthesis of the alkaloid itself.[49] A simple synthetic procedure devised by Schroeter [50] is based on the observation that spontaneous polymerization of cyanoacetyl chloride results in 2,4-dihydroxy-6-chloronicotinic acid nitrile (VIII). On methylation, this substance reacts in a pyridone form, yielding an N-methyl derivative (IX) from which, by dehalogenation (formation of ricininic acid III) and further methylation, ricinine is obtained.

VIII   IX   III   I
Chloronorricinine   Chlororicininic acid   Ricininic acid   Ricinine

Ricinine is mildly poisonous, but the toxic properties of castor beans appear to be due to a phytotoxin, ricin, of unknown nature.

[49] Späth and Koller, *Ber.*, **56**, 880, 2454 (1923); **58**, 2124 (1925).
[50] Schroeter, Seidler, Sulzbacher, and Kanitz, *Ber.*, **65**, 432 (1932).

PYRROLIDINE GROUP

Hygrine Alkaloids. Hygrine, $C_8H_{15}ON$, occurs in the leaves of the Peruvian coca shrub, *Erythroxylon coca*, from which it is obtained as an oily fraction along with some cuscohygrine, after the alkaloids of the cocaine group have been removed. Hygrine is one of the liquid alkaloids, and is optically active. The nitrogen atom carries a methyl group, and oxygen is present in ketone form.

The structural formula of hygrine is based upon the relationship to hygrinic (hygric) acid and upon synthesis. By oxidation with chromic acid, hygrine is converted to hygrinic acid, $C_5H_{10}NCOOH$, a monobasic acid that breaks down on dry distillation into carbon dioxide and N-methylpyrrolidine. The ease of decomposition indicates the α-position for the carboxyl group; this was shown by Willstätter's hygrinic acid synthesis [51] to be correct.

$$
\begin{array}{cc}
CH_2-CH_2 & CH_2-CH_2 \\
| \quad | & | \quad | \\
CH_2 \quad CHCOOH & CH_2 \quad CHCH_2COCH_3 \\
\diagdown N \diagup & \diagdown N \diagup \\
| & | \\
CH_3 & CH_3 \\
\text{I. Hygrinic acid} & \text{II. Hygrine}
\end{array}
$$

Hygrine differs from hygrinic acid by a C_3H_5O group in place of carboxyl. For this group only the forms $—COC_2H_5$ and $—CH_2COCH_3$ are possible; the choice of the latter rests on the (racemic) hygrine synthesis of Hess.[52] Pyrrylmagnesium bromide was treated with propylene oxide, yielding 1-α-pyrryl-2-propanol, from which the corresponding pyrrolidine derivative (IV) was obtained by catalytic hydrogenation.

$$
C_4H_4NMgBr + CH_2-CHCH_3 \xrightarrow{(+H_2O)} \underset{\overset{|}{H}}{\boxed{}}_N CH_2CHOHCH_3 \xrightarrow{4H}
$$

III. 1-α-Pyrryl-2-propanol

$$
\begin{array}{ccc}
CH_2-CH_2 & & CH_2-CH_2 \\
| \quad | & \xrightarrow{HCHO} & | \quad | \\
CH_2 \quad CHCH_2CHOHCH_3 & & CH_2 \quad CHCH_2COCH_3 \\
\diagdown N \diagup & & \diagdown N \diagup \\
H & & | \\
& & CH_3
\end{array}
$$

IV. 1-α-Pyrrolidyl-2-propanol   II. d,l-Hygrine

[51] Willstätter, *Ber.*, **33**, 1160 (1900).
[52] Hess, *Ber.*, **46**, 3113, 4104 (1913).

The pyrrolidine imino group was methylated with formaldehyde by the Eschweiler reaction, whereby the secondary alcoholic group unexpectedly contributed its hydrogen atoms toward formation of the methyl group, and appeared as the hygrine carbonyl in the end product.*

Attachment of the hygrine side chain to the α-carbon atom of the pyrrolidine nucleus suggests a phytochemical relationship between hygrine and tropinone.[51]

$$
\begin{array}{ccc}
\text{CH}_2\!\!-\!\!-\!\!-\text{CH}\!\!-\!\!-\!\!-\text{CH}_2 & \quad & \text{CH}_2\!\!-\!\!-\!\!-\text{CH}\!\!-\!\!-\!\!-\text{CH}_2 \\
| \qquad | & & | \qquad | \\
\text{N}\!\!-\!\!\text{CH}_3 \quad \text{CO} & & \text{N}\!\!-\!\!\text{CH}_3 \quad \text{CO} \\
| \qquad | & & | \qquad | \\
\text{CH}_2\!\!-\!\!-\!\!-\text{CH}_2 \quad \text{CH}_3 & & \text{CH}_2\!\!-\!\!-\!\!-\text{CH}\!\!-\!\!-\!\!-\text{CH}_2 \\
\text{Hygrine} & & \text{Tropinone}
\end{array}
$$

Stachydrine is the methylbetaine of hygrinic acid and occurs rather widely in nature (chrysanthemum, alfalfa, citrus, and *Stachys* species).

Cuscohygrine, $C_{13}H_{24}ON_2$, is found chiefly in the so-called cusco coca leaves. It is an optically inactive diacid base, closely related to hygrine. The action of alcoholic alkali degrades it in part to hygrine, and like hygrine it can be oxidized to hygrinic acid. Two formulas have been proposed for cuscohygrine:

$$
\begin{array}{ccc}
\text{CH}_2\!\!-\!\!\text{CH}_2 & \text{CH}_2\!\!-\!\!\text{CH}_2 & \text{CH}_2\!\!-\!\!\text{CH}_2 \qquad \text{CH}_2\!\!-\!\!\text{CH}_2 \\
| \quad | & | \quad | & | \quad | \qquad\quad | \quad | \\
\text{CH}_2 \ \ \text{CH}\!\!-\!\!\text{CH}_2\text{COCH}_2\!\!-\!\!\text{CH} \ \ \text{CH}_2 & & \text{CH}_2 \ \ \text{CH}\!\!-\!\!\text{CH}\!\!-\!\!\text{CH} \ \ \text{CH}_2 \\
\diagdown\!\text{N}\!\diagup & \diagdown\!\text{N}\!\diagup & \diagdown\!\text{N}\!\diagup \quad | \quad \diagdown\!\text{N}\!\diagup \\
| & | & | \quad\ \ \text{CO} \quad\ | \\
\text{CH}_3 & \text{CH}_3 & \text{CH}_3 \quad | \quad \text{CH}_3 \\
& & \qquad\quad \text{CH}_3 \\
\text{V} & \text{Cuscohygrine} & \text{VI}
\end{array}
$$

Formula V is in better accord with the formation of undecane and 6-undecanol in the Hofmann degradation of dihydrocuscohygrine (i.e., cuscohygrine reduced at the carbonyl group), which indicates an unbranched chain of eleven carbon atoms. Formula VI was advanced by Hess to explain the appearance of homohygrinic acid (N-methyl-α-pyrrolidylacetic acid) in Traube's reaction† and of a supposed di-(N-methyl-α-pyrrolidyl)methane in decompositions of cuscohygrine hydra-

---

* The Eschweiler method for the methylation of primary or secondary amines consists in heating the amine with formaldehyde. The hydrogen necessary for the formation of the methyl group is supplied by the excess formaldehyde, which is oxidized to formic acid or to carbon dioxide. [Eschweiler, *Ber.*, **38**, 880 (1905); Hess, *Ber.*, **46**, 4104, footnote (1913).]

† Traube's reaction [*Ann.*, **300**, 81 (1898)] depends upon the ability of the hydrogen in methyl, methylene, or methenyl groups adjacent to a carbonyl group to react with nitric oxide in the presence of sodium ethoxide. From the number of moles of nitric oxide which react, and the nature of the products of subsequent hydrolysis, it is possible to distinguish between —COCH₃, —COCH₂—, and —COCH= groups.

zone.[43] Barring the possibility of a rearrangement during the Hofmann degradation, formula V seems the more probable for cuscohygrine.

PYRIDINE-PYRROLIDINE AND DIPYRIDINE GROUP

Tobacco and Anabasis Alkaloids. The alkaloid nicotine, from *Nicotiana tabacum*, occupies a position of great commercial importance. The annual world production of tobacco for human consumption and insecticidal use is more than two million tons, corresponding to about sixty or seventy thousand tons of nicotine alkaloid. The base is combined in the plant with malic and citric acids and may be isolated by extracting the powdered leaves and stems with water, liberating the alkaloids with alkali, and distilling with steam. The crude nicotine is purified through the oxalate. It is also commercial practice to extract systematically with trichloroethylene a mixture of tobacco refuse, milk of lime, and sodium hydroxide. The solution is concentrated in a vacuum and extracted with dilute sulfuric acid, from which the nicotine is liberated with alkali and extracted into ether-petroleum ether mixture. Distillation of this solution under nitrogen yields nearly pure nicotine.

Nicotine is a strongly basic levorotatory liquid, miscible with water below 60° in the form of a hydrate, and above 210°. Its structure has been shown both by degradation and synthesis. Oxidation with a variety of agents leads to nicotinic acid, β-pyridinecarboxylic acid (IV). The alkaloid must therefore be a pyridine derivative carrying a $C_5H_{10}N$ group in the β-position. This side chain cannot consist of a piperidine nucleus, for nicotine behaves as a bitertiary base, that is, contains no >NH group; furthermore, the Herzig and Meyer determination shows the presence of an N-methyl group. The methyl group cannot be attached to the pyridine nitrogen atom, so the $C_5H_{10}N$ group is resolved into $C_4H_7NCH_3$. These facts are best explained by a pyridine-N-methylpyrrolidine system.[44]

I. Nicotine

The linkage of the pyridine ring in the α-position of the pyrrolidine nucleus was shown by bromine degradation. Nicotine, on treatment

[43] Hess and Bappert, *Ann.*, **441**, 137 (1925); Sohl and Shriner, *J. Am. Chem. Soc.*, **55**, 3528 (1933); Hess and Fink, *Ber.*, **53**, 781 (1920).

[44] Pinner, *Ber.*, **26**, 292 (1893).

with bromine, yields a dibromoketone, dibromoticonine. With barium hydroxide, dibromoticonine breaks down to nicotinic acid, malonic acid, and methylamine.

| II. Dibromoticonine | III Intermediate | IV. Nicotinic acid | Malonic acid |

The appearance of the three-carbon acid, malonic, shows that the carbon atom appearing in the carboxyl group of nicotinic acid must be the end atom of a chain of four carbons, which is possible only if the pyrrolidine ring is linked through an α-position.

With weak oxidizing agents the methylpyrrolidine nucleus of nicotine is attacked, resulting in nicotyrine, a base that appears as an intermedi-

V. Nicotyrine

ate in the nicotine synthesis of Pictet.[55] This synthesis has its starting point in a reaction parallel to the formation of pyrrole through dry distillation of ammonium mucate.

$$\begin{matrix} CHOH—CHOH—COONH_4 \\ | \\ CHOH—CHOH—COONH_4 \end{matrix} \rightarrow \begin{matrix} CH=CH \\ | \qquad\quad \rangle NH + NH_3 + 4H_2O + 2CO_2 \\ CH=CH \end{matrix}$$

By using the mucate of β-aminopyridine, that is, by substituting the pyridine group for one hydrogen of ammonia in the above reaction, Pictet obtained β-pyridyl-N-pyrrole. Pyrroles carrying carbon substituents on nitrogen undergo on heating a rearrangement that involves a shift of the group from nitrogen to the pyrrole α-position, and β-pyridyl-N-pyrrole was converted by this method to β-pyridyl-α-pyrrole.

$$\begin{matrix} CHOH—CHOH—COONH_3—C_5H_4N \\ | \\ CHOH—CHOH—COONH_3—C_5H_4N \end{matrix} \rightarrow$$

β-Aminopyridine mucate

VI. β-Pyridyl-N-pyrrole

[55] Pictet and Rotschy, Ber., 37, 1225 (1904).

VII. β-Pyridyl-α-pyrrole VIII. Nicotyrine methiodide V. Nicotyrine

The potassium salt of the latter substance, when heated with methyl iodide, yielded nicotyrine methiodide, from which nicotyrine (V) could be obtained by heating with lime. By halogenation and reduction with tin and acid, the methylpyrryl nucleus alone of nicotyrine was hydrogenated, and the resulting d,l-nicotine could be resolved with tartaric acid into d-nicotine and the familiar l-nicotine. The reduction of nicotyrine can also be accomplished with catalytic hydrogen.[56]

The Pictet synthesis involves violent and complicated reactions which are of doubtful value for structural proof. A more transparent synthesis by Späth [57] confirms, however, the accepted structure. Nicotinic ethyl ester was condensed with N-methylpyrrolidone, resulting in the ketone (XI). The pyrrolidonyl ring of this ketone suffered scission and loss of carbon dioxide when heated with fuming hydrochloric acid, yielding the open-chain amino ketone (XII). By reduction of the amino ketone to the corresponding alcohol, iodination, and elimination of hydrogen iodide, the N-methylpyrrolidine ring of nicotine was constructed in a manner that leaves no question as to its point of attachment.

IX. Nicotinic ethyl ester X. N-Methyl-pyrrolidone XI. β-Pyridyl β-N-methyl-α-pyrrolidonyl ketone

XII. β-Pyridyl γ-methyl-amino-α-propyl ketone I. Nicotine

[56] Späth and Kuffner, Ber., 68, 494 (1935).
[57] Späth and Bretschneider, Ber., 61, 327 (1928).

The synthesis of α-nicotine, α-pyridyl-α-N-methylpyrrolidine, which is not known to occur naturally, has also been accomplished.[58]

Pictet and Noga have described nicoteine, isonicoteine, nicotoine, nicotimine, and nicotelline as minor alkaloids. The most abundant of these, nicoteine, was shown by Ehrenstein [59] to be a mixture of two alkaloids, so that the existence of the rarer members as individuals may well be doubted. The so-called nicoteine was separated by fractional crystallization of the picrate into nornicotine and l-β-pyridyl-α-piperidine. The latter substance has the formula that had already been assigned without adequate evidence to Pictet's nicotimine. Nornicotine can be prepared by demethylation of nicotine, or by total synthesis from pyridine.[60, 61] Both d- and l-nornicotine have been found in tobacco. The constituents of tobacco smoke have been extensively studied. At least eight bases appear to be present, of which myosmine and the three sokratines are responsible for the aroma. Myosmine has been shown to be a β-pyridyl-α-pyrroline.[62]

Nornicotine   Myosmine   Nicotimine (Anabasine) (β-Pyridyl-α-piperidine)

As the chief alkaloid of the poisonous Asiatic plant *Anabasis aphylla*, Orechoff [63] isolated the base anabasine. This substance is identical with the above-mentioned l-β-pyridyl-α-piperidine. Its constitution could be shown by oxidation to nicotinic acid, and by dehydrogenation to α,β-bipyridyl.

Nicotine is one of the most poisonous alkaloids, the fatal dose for man being in the neighborhood of 40 mg. In smaller amounts it causes dizziness, perspiration, salivation, and intestinal disturbances. d-Nicotine shows only one-half the physiological activity of natural l-nicotine. Anabasine, like nicotine, is very poisonous and has high insecticidal action.

[58] Craig, *J. Am. Chem. Soc.*, **56**, 1144 (1934).
[59] Ehrenstein, *Arch. Pharm.*, **269**, 627 (1931).
[60] Craig, *J. Am. Chem. Soc.*, **55**, 2854 (1933).
[61] Spāth, Marion, and Zajic, *Ber.*, **69**, 251 (1936).
[62] Spāth, Wenusch, and Zajic, *Ber.*, **69**, 393 (1936); Spāth and Mamoli, *Ber.*, **69**, 757 (1936).
[63] Orechoff and Menschikoff, *Ber.*, **64**, 266 (1931).

CONDENSED PIPERIDINE-PYRROLIDINE GROUP. BELLADONNA AND COCA ALKALOIDS

Belladonna Alkaloids. The roots and leaves of a number of solanaceous plants, notably belladonna (*Atropa belladonna*), henbane (*Hyoscyamus niger*), the thorn apple (*Datura stramonium*), and some *Duboisia* and *Scopolia* species, are rich in a series of therapeutically important alkaloids. Hyoscine and hyoscyamine occur in nearly all these plants, accompanied occasionally by atropine, apoatropine, norhyoscyamine, belladonnine, and meteloidine. The solanaceous plants are notorious hallucinants, the drugs of fanaticism. The group furnished the "sorcerer's drugs" of the Middle Ages, and *Hyoscyamus*, *Datura*, and *Duboisia* leaves are today smoked, chewed, or consumed in decoctions in parts of Egypt, India, South America, and Australia for the hallucinations and frenzy that they produce.[45]

Atropine, $C_{17}H_{23}O_3N$, is the racemic form of hyoscyamine. Although it is undoubtedly formed to a large extent from the latter base during isolation and purification, it has also been shown to exist as such in the plant. All the atropine of commerce is prepared by racemization of hyoscyamine with dilute alkali. Atropine is an ester; on hydrolysis it yields tropic acid, $C_9H_{10}O_3$, and tropine, $C_8H_{15}ON$.

Determination of the structure of tropic acid offered little difficulty. This acid is converted by dehydrating agents to α-phenylacrylic acid (atropic acid), a type of change characteristic of β-hydroxy acids, but not of α-hydroxy acids. Tropic acid must therefore possess formula II, for the only alternative (III) is that shown by synthesis to belong to atrolactic acid.

I. Atropic acid   II. Tropic acid   III. Atrolactic acid

Tropic acid was synthesized by Ladenburg from acetophenone.[44] It contains an asymmetric carbon atom, and to it hyoscyamine owes its optical activity.

The basic portion obtained from the hydrolysis of atropine or hyoscyamine, namely tropine, is optically inactive. The two asymmetric carbon atoms in positions 1 and 5 compensate, and the 3-carbon atom is pseudoasymmetric. The molecule is symmetrical, and cannot be resolved into active components. The tropine structural formula was developed chiefly by Merling and by Willstätter on the basis of the fol-

44 Ladenburg and co-workers, *Ber.*, **13**, 2041 (1880); **22**, 2590 (1889).

lowing evidence. Tropine is a tertiary base containing an N-methyl group and an alcoholic hydroxyl. By gentle oxidation it is converted to a ketone, tropinone.

$$
\begin{array}{cccc}
CH_2\!\!-\!\!-\!\!-\!\!CH\!\!-\!\!-\!\!-\!\!CH_2 \\
|7 \quad |1 \quad 2| \\
| \quad\quad NCH_3 \; 3\,CHOH \\
|6 \quad |5 \quad 4| \\
CH_2\!\!-\!\!-\!\!-\!\!CH\!\!-\!\!-\!\!-\!\!CH_2 \\
\text{IV. Tropine}
\end{array}
\qquad
\begin{array}{cccc}
CH_2\!\!-\!\!-\!\!-\!\!CH\!\!-\!\!-\!\!-\!\!CH_2 \\
| \quad\quad | \quad\quad | \\
| \quad\quad NCH_3 \quad CO \\
| \quad\quad | \quad\quad | \\
CH_2\!\!-\!\!-\!\!-\!\!CH\!\!-\!\!-\!\!-\!\!CH_2 \\
\text{V. Tropinone}
\end{array}
$$

This ketone forms diisonitroso and dibenzylidene derivatives, and therefore has two methylene groups adjacent to the carbonyl. Tropinone gives on further oxidation a dicarboxylic acid (tropinic acid, VI) with the same number of carbon atoms; hence the ketone group cannot be in a side chain. Application of the exhaustive methylation process to tropinic acid yields pimelic acid (compare the degradation of methylgranatic acid, p. 1182) containing a straight seven-carbon chain. Oxidation of tropinic acid, on the other hand, results in N-methylsuccinimide, whereby the position of nitrogen is shown and the pyrrolidine ring is revealed.

$$
\begin{array}{c}
CH_2\!\!-\!\!-\!\!CH\!\!-\!\!-\!\!CH_2 \\
| \quad\quad | \quad\quad | \\
| \quad\quad NCH_3 \; COOH \\
| \quad\quad | \\
CH_2\!\!-\!\!-\!\!CH\!\!-\!\!-\!\!COOH \\
\text{VI. Tropinic acid}
\end{array}
\qquad
\begin{array}{c}
CH_2\!\!-\!\!CH_2\!\!-\!\!CH_2 \\
| \quad\quad\quad | \\
| \quad\quad\quad COOH \\
| \\
CH_2\!\!-\!\!CH_2\!\!-\!\!COOH \\
\text{VII. Pimelic acid}
\end{array}
\qquad
\begin{array}{c}
CH_2\!\!-\!\!C\diagdown^{O} \\
| \quad\quad\quad\diagup NCH_3 \\
CH_2\!\!-\!\!C\diagdown_{O} \\
\text{VIII. N-Methyl-} \\
\text{succinimide}
\end{array}
$$

Tropine consists therefore of a fused piperidine-pyrrolidine skeleton in which the two ring systems have nitrogen and two carbon atoms in common. The esters of tropine, of which many have been prepared, are called tropeines; the most important synthetic ester is mandelyltropeine, a powerful mydriatic known as homatropine. Atropine is *d,l*-tropyltropeine, hyoscyamine is the *levo* form.

$$
\begin{array}{c}
CH_2\!\!-\!\!-\!\!CH\!\!-\!\!-\!\!CH_2 \qquad CH_2OH \\
| \quad\quad | \quad\quad |\diagup H \quad\quad | \\
| \quad\quad NCH_3 \; C\!\!-\!\!\diagup O\!\!-\!\!CO\!\!-\!\!CHC_6H_5 \\
| \quad\quad | \quad\quad | \\
CH_2\!\!-\!\!-\!\!CH\!\!-\!\!-\!\!CH_2 \\
\text{IX. Atropine, Hyoscyamine}
\end{array}
$$

The preparation of atropine from tropine and tropic acid was accomplished by Ladenburg in 1879,[65] and both components were later synthesized. For the preparation of tropine, Willstätter[66] chose suberone as the starting point. This ketone was converted to cycloheptene through

---

[65] Ladenburg, *Ber.*, **12**, 941 (1879).

[66] Willstätter, *Ber.*, **34**, 131, 3163 (1901); *Ann.*, **326**, 23 (1903).

suberol and suberyl iodide, or by exhaustive methylation of the amine resulting from reduction of suberone oxime.

$$CH_2{-}CH_2{-}CH_2$$
$$CH_2{-}CH_2{-}CH_2{\bigg>}CO \rightarrow$$
X. Suberone

$$-CH_2$$
$$-CH_2{\bigg>}CHOH \rightarrow$$
XI. Suberol

$$-CH_2$$
$$-CH_2{\bigg>}CHI \rightarrow$$
XII. Suberyl iodide

$$CH_2{-}CH_2{-}CH_2$$
$$CH_2{-}CH_2{-}CH{\bigg>}CH \rightarrow$$
XIII. Cycloheptene

$$CH_2{-}CH_2{-}CH_2$$
$$CH_2{-}CH_2{-}CHBr{\bigg>}CHBr \rightarrow$$
XIV. Cycloheptene dibromide

$$CH_2{-}CH_2{-}CH_2$$
$$CH_2{-}CH{=}CH{\bigg>}CHN(CH_3)_2 \rightarrow$$
XV. Dimethylaminocyclo-heptene

$$CH_2{-}CH_2{-}CH$$
$$CH_2{-}CH{=}CH{\bigg>}CH$$
XVI. Cycloheptadiene

A second unsaturated linkage was introduced by the following ingenious device. Cycloheptene dibromide was treated with dimethylamine, yielding a tertiary amine, the methiodide of which could be degraded by Hofmann's method (p. 1172) to trimethylamine and cycloheptadiene. Cycloheptadiene dibromide suffered loss of hydrobromic acid in the presence of quinoline to give cycloheptatriene (XVII), identical with the tropilidene already known from the degradation of tropine. By addition of hydrogen bromide and subsequent reaction with dimethylamine, cycloheptatriene was converted to the amine, α-methyltropidine. Partial reduction of α-methyltropidine, addition of bromine, and rearrangement of the dibromo compound led to 2-bromotropane methobromide, containing the desired nitrogen bridge.

$$CH_2{-}CH{=}CH$$
$$\qquad CH \rightarrow$$
$$CH{=}CH{-}CH$$
XVII. Cycloheptatriene

$$CH_2{-}CH{-}\!\!-\!\!-CH$$
$$\quad N(CH_3)_2\ CH \rightarrow$$
$$CH_2{-}CH{=}\!\!=\!\!CH$$
XVIII. α-Methyltropidine

$$CH_2{-}CH{-}\!\!-\!\!-CH_2$$
$$\quad N(CH_3)_2\ CH_2 \rightarrow$$
$$CH_2{-}CHBr{-}\!\!-\!\!-CHBr$$
XIX. Methyltropane dibromide

$$CH_2{-}CH{-}\!\!-\!\!-CH_2$$
$$\quad N{\big\langle}^{(CH_3)_2}_{\ Br}\ CH_2 \rightarrow$$
$$CH_2{-}CH{-}\!\!-\!\!-CHBr$$
XX. 2-Bromotropane methobromide

$$CH_2{-}CH{-}\!\!-\!\!-CH_2$$
$$\quad NCH_3\ CH \rightarrow$$
$$CH_2{-}CH{-}\!\!-\!\!-CH$$
XXI. Tropidine

$$CH_2{-}CH{-}\!\!-\!\!-CH_2$$
$$\quad NCH_3\ CHBr \rightarrow$$
$$CH_2{-}CH{-}\!\!-\!\!-CH_2$$
XXII. 3-Bromotropane

$$CH_2{-}CH{-}\!\!-\!\!-CH_2$$
$$\quad NCH_3\ CHOH \rightarrow$$
$$CH_2{-}CH{-}\!\!-\!\!-CH_2$$
XXIII. Pseudotropine

$$CH_2{-}CH{-}\!\!-\!\!-CH_2$$
$$\quad NCH_3\ CO \rightarrow$$
$$CH_2{-}CH{-}\!\!-\!\!-CH_2$$
V. Tropinone

$$CH_2{-}CH{-}\!\!-\!\!-CH_2$$
$$\quad NCH_3\ C{\big\langle}^{OH}_{H}$$
$$CH_2{-}CH{-}\!\!-\!\!-CH_2$$
IV. Tropine

From 2-bromotropane methobromide, hydrogen bromide was eliminated by the action of alkali, giving tropidine methobromide. Through the

usual steps for converting a quaternary halide to the tertiary base, tropine methobromide was transformed to the methochloride and the latter distilled; tropidine (XXI) and methyl chloride were the products. From tropidine and hydrobromic acid, 3-bromotropane was obtained, which on hydrolysis with dilute sulfuric acid yielded the stereoisomer, pseudotropine, instead of the expected tropine. Pseudotropine was therefore oxidized to tropinone, which could then be reduced to tropine.

Another synthesis of extraordinary simplicity and elegance was devised by Robinson.[67] Succinaldehyde, methylamine, and acetone (or better, calcium acetonedicarboxylate), on standing in alkaline solution, gave tropinone.

$$
\begin{array}{ccccc}
CH_2\!\!-\!\!CHO & HCH_2 & CH_2\!\!-\!\!CH\!\!-\!\!CH_2 \\
| & | & | & | & | \\
& H_2NCH_3 \quad CO & \rightarrow & NCH_3 \quad CO \\
| & | & | & | & | \\
CH_2\!\!-\!\!CHO & HCH_2 & CH_2\!\!-\!\!CH\!\!-\!\!CH_2
\end{array}
$$

A later synthesis by Willstätter likewise has its starting point in acetonedicarboxylic acid.[68] The success of Schöpf in carrying out the Robinson synthesis under physiological conditions (p. 1253) makes it seem probable that the plant employs a similar method.

Scopolamine, also known as hyoscine, is an ester of the optically inactive amino alcohol scopine (XXV) with *l*-tropic acid.

$$
\begin{array}{ccc}
CH\!\!-\!\!CH\!\!-\!\!CH_2 \\
/ \ | \quad | \\
O \ | \quad NCH_3 \quad CH\!\!-\!\!OCO\!\!-\!\!CH\!\!-\!\!C_6H_5 \\
\backslash \ | \quad | \quad | \\
CH\!\!-\!\!CH\!\!-\!\!CH_2 \quad CH_2OH
\end{array}
$$

XXIV. Scopolamine

Scopolamine is levorotatory, but is racemized with great ease, the *d,l*-form being atroscine. By hydrolysis of scopolamine under very mild conditions, with pancreatic lipase or with Michaelis' buffer solution, Willstätter [69] was able to obtain scopine itself (XXV). Scopine undergoes rearrangement with great ease into scopoline (oscine), which is the basic product obtained when scopolamine is hydrolyzed in the usual way.

$$
\begin{array}{cc}
CH\!\!-\!\!CH\!\!-\!\!CH_2 & HO\!\!-\!\!CH\!\!-\!\!CH\!\!-\!\!CH_2 \\
/ \ | \quad | & | \quad NCH_3 \quad CH\!\!- \\
O \ | \quad NCH_3 \quad CHOH & | \quad | \quad | \\
\backslash \ | \quad | & CH\!\!-\!\!CH\!\!-\!\!CH_2 \\
CH\!\!-\!\!CH\!\!-\!\!CH_2 & O\!\!-
\end{array}
$$

XXV. Scopine   XXVI. Scopoline

[67] Robinson, *J. Chem. Soc.*, **111**, 762 (1917).
[68] Willstätter and Pfannenstiel, *Ann.*, **422**, 1 (1921).
[69] Willstätter and Berner, *Ber.*, **56**, 1079 (1923).

Apoatropine (atropamine), found in belladonna root, can also be obtained from atropine or hyoscyamine by the action of dehydrating agents. It is an ester of tropine and atropic acid (α-phenylacrylic acid, I), and was obtained by combining these two substances before it was found in nature.

Belladonnine, an isomer or polymer of apoatropine, was first isolated from belladonna root. It can be prepared by the action of hot baryta water on apoatropine. By vigorous hydrolysis with hydrochloric acid it can be broken down to 3-chlorotropane, showing that it contains the tropine nucleus.[70]

Norhyoscyamine (pseudohyoscyamine), from *Duboisia*, *Scopolia*, and *Datura* species, consists of tropic acid esterified with nortropine (tropigenine), a tropine containing the $>NH$ group in place of $>NCH_3$. Norhyoscyamine can be racemized easily to the corresponding *d,l*-form, noratropine. The latter probably does not occur in nature but appears as a result of racemization of the active form.

Meteloidine is a rare alkaloid of *Datura meteloides* and is an ester of tiglic acid with teloidine. Teloidine is believed to be dihydroxytropine and is closely related to scopine and scopoline.[71]

$$HO-CH-CH-CH_2$$
$$| \quad | \quad |$$
$$NCH_3 \quad CHOCOC=CHCH_3$$
$$| \quad | \quad | \quad |$$
$$HO-CH-CH-CH_2 \quad CH_3$$

XXVII. Meteloidine

The alkaloids of the atropine group dilate the pupil and paralyze the accommodation muscles of the eye. Atropine thus finds extensive use in ophthalmic practice. It has a stimulating action on the cerebrum and respiratory center. Hyoscyamine resembles atropine, but is stronger in action. Scopolamine has a stupefying effect, and is often used in combination with morphine, as well as in the treatment of morphinism.

Coca Alkaloids. The leaves of *Erythroxylon coca*, which have been used as a stimulant by the South American Indians for centuries, contain as the active principle cocaine, with which is associated a number of other alkaloids of closely related structure. The great importance of cocaine in medical practice has resulted in extensive cultivation of several *Erythroxylon* species in Peru, Bolivia, Java, and Ceylon. The legitimate world production of cocaine dropped from 6434 kg. in 1929 to 4010 kg. in 1933, probably because of increasing use of substitutes and more effective control of international trade. The League of Nations report for 1939

[70] Polonovski, *Bull. soc. chim.*, [4] **45**, 304 (1929).
[71] King, *J. Chem. Soc.*, **115**, 476 (1919).

shows a world production of 3045 kg. The illegitimate production is large, being unofficially estimated at 15,000 to 20,000 kg.

Cocaine, $C_{17}H_{21}O_4N$, is an ester; it is hydrolyzed by boiling water into benzoyl-l-ecgonine and methanol, or by acids and alkalies into l-ecgonine, benzoic acid, and methanol.

$$C_{17}H_{21}O_4N + 2H_2O \rightarrow C_9H_{15}O_3N + C_7H_6O_2 + CH_3OH$$
$$\text{Cocaine} \qquad\qquad \text{l-Ecgonine} \quad \text{Benzoic acid}$$

This process may be reversed, and in commercial practice it is customary, especially with Java leaves, to hydrolyze all the ecgonine derivatives present (including the cocaine) to ecgonine. This base is then benzoylated with benzoic anhydride and the benzoylecgonine esterified with methanol and acid; methylation followed by benzoylation is also employed. An amount of cocaine considerably greater than that originally present in the leaves is thus obtained.

Ecgonine is a tertiary base and forms quaternary halides with one mole of alkyl halide. The presence of the carboxyl and alcoholic hydroxyl groups is evident from the esterification reactions mentioned. The structural skeleton of ecgonine was disclosed through relationships to tropine. With dehydrating agents ecgonine passes into anhydroecgonine, an unsaturated acid, which decomposes in the presence of hydrochloric acid at 280° into carbon dioxide and tropidine. The constitution of tropidine is discussed under the atropine group.

| I. Ecgonine | II. Anhydroecgonine | III. Tropidine |

The position of the hydroxyl and carboxyl groups of ecgonine rests on the following considerations. Ecgonine, like tropine, yields tropinone by chromic acid oxidation, and since the carbonyl can scarcely be formed except by oxidation of the alcoholic group, ecgonine must have the hydroxyl in the same position as has tropine. Willstätter [72] was able to demonstrate that the oxidation of ecgonine proceeds through an intermediate keto acid, which loses carbon dioxide with great ease. The appearance of the keto acid excludes the possibility that the carboxyl and hydroxyl groups occupy the same carbon atom. Location of the carboxyl in a γ-position to the hydroxyl is not in accord with the instability of the intermediate acid, hence only a β-position comes into consideration.

[72] Willstätter and Müller, *Ber.*, **31**, 2655 (1898).

Tropane, the parent substance of the ecgonine series, contains two asymmetric carbon atoms, C-1 and C-5, to which the nitrogen bridge is attached. These asymmetric atoms are equal and opposite in their rotatory power, which results in internal compensation. Tropane is a meso-form, and has a symmetrical molecule.

IV   V

When a hydroxyl group appears on C-3 (as in tropine), the symmetry of the molecule is not destroyed; C-3 in tropine is pseudoasymmetric. The hydroxyl may occupy two positions with reference to the nitrogen ring, giving rise to tropine and pseudotropine, which are *cis-trans* isomers and not optical opposites.

VI. Tropane

VII. Tropine, Pseudotropine

The presence of a carboxyl group in the tropine or pseudotropine framework destroys the symmetry of the molecule. In addition to the new asymmetric atom carrying the carboxyl, carbon-3 now becomes truly asymmetric, and the asymmetric atoms 1 and 5, carrying the nitrogen bridge, become dissimilar. Sixteen optically active isomers would be expected, but C-1 and C-5 can have only one configuration because of the restriction imposed by the nitrogen bridge. Therefore only eight optical isomers and four racemates can exist.[73, 74] A vertical projection of the three tropane ring planes of Figs. IV and V shows these isomers thus:

Tropane-3-cis-ol-2-cis-carboxylic acid

[73] Willstätter and Bommer, *Ann.*, **422**, 15 (1921).
[74] Mannich, *Arch. Pharm.*, **272**, 324 (1934).

Tropane-3-*cis*-ol-2-*trans*-carboxylic acid

Tropane-3-*trans*-ol-2-*cis*-carboxylic acid

Tropane-3-*trans*-ol-2-*trans*-carboxylic acid

The synthesis of ecgonine through reduction of tropinonecarboxylic ester (VIII to IX) led to the isolation of a racemic ecgonine methyl ester belonging to the pseudo series. On resolution the racemate yielded *d*-pseudoecgonine methyl ester and the corresponding *l*-form, from which *d*-pseudococaine (the drug psicain) and *l*-pseudococaine, respectively, could be prepared by benzoylation. In the same reduction two other racemates were formed. One of these has not been resolved. The other belongs to the tropine series and after benzoylation was resolved into *d*-cocaine and the naturally occurring *l*-cocaine.[75]

CH₂—CHO

CH₂—CHO

Succinaldehyde

H₂NCH₃

Methylamine

CH₂COOCH₃

CO

CH₂COOH

Acetonedicarboxylic acid
monomethyl ester

→

VIII. Tropinonecarboxylic ester

IX. Racemic ecgonine and pseudoecgonine ester

→

α-Ecgonine is an isomer of ecgonine in which both the hydroxyl and carboxyl groups are located on carbon-3. It was prepared by addi-

[75] Willstätter, Wolfes, and Mäder, *Ann.*, **434**, 111 (1923).

tion of hydrogen cyanide to tropinone and hydrolysis of the resulting cyanohydrin.

l-Cinnamylcocaine is the ester of methyl-*l*-ecgonine with cinnamic acid. It is the chief alkaloid of Java coca leaves (*Erythroxylon truxillense*).

α-Truxilline, also known as cocamine or γ-isatropylcocaine, is an ester of two molecules of methyl-*l*-ecgonine with one molecule of α-truxillic acid. β-Truxilline (isococamine or δ-isatropylcocaine) is the analogous ester with β-truxillic acid. Both truxillines are present in Peruvian leaves.

Tropacocaine, which occurs in Java and Peruvian leaves, does not belong to the ecgonine series, but is a tropa alkaloid. On hydrolysis it yields benzoic acid and pseudotropine. In addition to the above-mentioned alkaloids, coca also contains small amounts of benzoyl-*l*-ecgonine, and the hygrine alkaloids, which have already been described.

Cocaine is an exceedingly valuable therapeutic agent because of its paralyzing effect on sensory nerve endings, with which is combined a local vasoconstriction. The latter action results in prolongation of the anesthesia by diminishing the speed of absorption; the delayed absorption likewise decreases systemic toxicity by permitting gradual destruction of the drug. Cocaine causes dilation of the pupils by central and peripheral stimulation of the pupillo-dilator mechanism. The relatively high toxicity of cocaine and its ability to produce a condition of euphoria, often leading to habituation, have resulted in the synthesis of numerous substitutes, as novocaine (the *p*-aminobenzoyl derivative of diethylaminoethanol), β-eucaine (benzoylvinyl diacetone alkamine hydrochloride), and psicain (*d*-pseudococaine acid tartrate). Tropacocaine is said to be more effective than cocaine as a local anesthetic, but has a disadvantageous hyperemic action.

QUINOLINE GROUP. CINCHONA AND ANGOSTURA ALKALOIDS

Cinchona Alkaloids.[76] Quinine and cinchonine, together with some twenty less important alkaloids of related structure, are found in the bark of several species of *Cinchona* and *Remijia*, trees native to high altitudes in the Andes. The bark of cultivated specimens of *C. ledgeriana* grafted on *C. succiruba*, as is customary in Java, may contain up to 10 per cent quinine, or total alkaloids up to 17 per cent. The famous *ledgeriana* graft "38n" contained in the trunk bark 18.5 per cent quinine (as sulfate) at the age of seven years. Commercial bark averages about 7 per cent quinine (sulfate). The world production of quinine follows the demand closely, and averages between 600,000 and 700,000 kg. of

[76] Commanducci, "Die Konstitution der Chinaalkaloide," in *Ahrens' Samml. chem. chem.-tech. Vorträge*, Vol. 16, p. 141 (1911).

quinine sulfate annually, 90 per cent of which is from the Dutch East Indies.[77] The bases are combined in the plant tissue with characteristic acids, chiefly quinic (tetrahydroxyhexahydrobenzoic), quinovic ($C_{30}H_{46}O_5$), and quinotannic (cinchotannic) acids. In commercial practice, the pulverized bark is steeped in slaked lime and sodium hydroxide, and extracted at 60° with aromatic solvents, as benzene or toluene. The mixed alkaloids are then extracted from the organic medium with dilute sulfuric acid. When this solution is brought nearly to the neutral point with sodium hydroxide, the sparingly soluble quinine sulfate, $Q_2H_2SO_4 \cdot 8H_2O$, separates. The less valuable minor alkaloids are then precipitated with excess alkali.

The parent alkaloid of the cinchona series, to which nearly half of the members are related, is cinchonine, $C_{19}H_{22}ON_2$. Cinchonine consists of a quinoline nucleus linked through a secondary alcoholic group to a quinuclidine ring system carrying a vinyl group.

I. Cinchonine

The usual analytical procedure shows the absence of methoxyl and methylimido groups in cinchonine. The presence of the secondary alcoholic group is evident from the results of acetylation and from the formation of the ketone, cinchoninone (VIII), in oxidation processes. The absorption of one mole of hydrogen by the catalytic method shows an ethylenic linkage. Cinchonine likewise adds halogens or halogen acids at the double bond. On treatment with hot concentrated alkali it is broken down to quinoline itself, or lepidine (4-methylquinoline), as well as to other quinoline and pyridine derivatives. Zinc dust distillation yields chiefly quinoline; vigorous oxidation results in cinchoninic acid.

II. Lepidine

III. Cinchoninic acid

The products of these degradations indicate a quinoline nucleus joined in the 4-position with a second heterocyclic ring. This other ring was designated for many years as "the second half," and substances

[77] "Chininum," Bureau Tot Bevordung van het Kinine-Gebruik, Amsterdam (1923).

derived from it were usually distinguished by the use of *loipon* or *meros* in their names. As fragments of the second half, Skraup was able to isolate after chromic acid oxidation the dibasic loiponic ($C_7H_{11}O_4N$) and cincholoiponic ($C_8H_{13}O_4N$) acids, and Koenigs found further a monobasic acid, meroquinene ($C_9H_{15}O_2N$).

COOH   CH$_2$COOH   CH$_2$COOH

IV. Loiponic acid   V. Cincholoiponic acid   VI. Meroquinene

Loiponic acid proved to be a labile form of the synthetically prepared hexahydrocinchomeronic acid (piperidine-3,4-dicarboxylic acid).[78] Cincholoiponic acid, a homolog of loiponic acid, can be converted by oxidation to loiponic acid, or by the action of hot sulfuric acid to γ-picoline (4-methylpyridine). The structure developed for cincholoiponic acid (V) from these observations was confirmed by Wohl's synthesis.[79]

The third product of cinchonine oxidation, meroquinene, furnished the key to the structure of the second half. On oxidation with permanganate, meroquinene yields cincholoiponic acid and formic acid, or by heating with hydrochloric acid it passes into 3-ethyl-4-methylpyridine. These facts indicate that the vinyl group is in the β-position to the nitrogen atom in the second half. All three oxidation products under discussion are secondary bases. The nitrogen atoms in cinchonine are tertiary, and since no N-methyl groups are present, the nitrogen in the second half must owe its tertiary nature to linkage in a condensed ring system. Oxidation of the second half to meroquinene involves oxidative scission of a ring, with formation of a carboxyl and an imino group.

VII. β-Vinylquinuclidine   VI. Meroquinene

A quinuclidine ring synthesis leading to β-ethylquinuclidine (which constitutes the second half in the natural alkaloids hydrocinchonine and hydroquinine) was accomplished by Koenigs.[80]

[78] Koenigs, *Ber.*, **30**, 1326 (1897).
[79] Wohl and Losanitsch, *Ber.*, **40**, 4698 (1907).
[80] Koenigs and Bernhart, *Ber.*, **38**, 3049 (1905).

The point of linkage of the quinuclidine group to the rest of the molecule was shown by Rabe [81] in studies on quininone and cinchoninone, the ketones resulting from gentle oxidation of quinine and cinchonine, respectively. These ketones have a CH group adjacent to, and activated by, the carbonyl group, and on treatment with amyl nitrite break down to quinoline acids (quininic and cinchoninic respectively) and an oxime (IX), 3-vinyl-8-oximinoquinuclidine.

$$CH_2\text{—}CH\text{—}CH\text{—}CH\text{=}CH_2 \qquad\qquad CH_2\text{—}CH\text{—}CH\text{—}CH\text{=}CH_2$$

$$\begin{array}{c} \text{CH}_2 \\ | \\ \text{CH}_2 \\ | \\ \text{R—CO—CH—N—CH}_2 \end{array} \quad \rightarrow \text{RCOOH} + \quad \begin{array}{c} \text{CH}_2 \\ | \\ \text{CH}_2 \\ | \\ \text{HON=C—N—CH}_2 \end{array}$$

VIII. Quininone, Cinchoninone   IX. 3-Vinyl-8-oximinoquinuclidine

The structure of IX was evident from the results of hydrolysis, which yielded hydroxylamine and meroquinene. The quinuclidine group must therefore be joined to the quinoline portion through a —CHOH— group attached to that carbon atom which appears as a carboxyl group in meroquinene.

Cinchonine and the other alkaloids of the cinchona group containing a vinyl group may be oxidized to the class designated as "tenines." Cinchotenine has a carboxyl group in place of the β-vinyl residue in cinchonine; from quinine and cupreine, quitenine and cuprotenine are obtained.

The cinchona alkaloids are further characterized by the ease with which they undergo isomerization. Of the seventeen or more cinchonine isomers that have been described by various investigators, at least eight are individuals. The most important of these are cinchotoxine and the natural alkaloid cinchonidine, which also results from treatment of cinchonine with alkali. Cinchotoxine is a rearrangement product obtained by the action of heat on cinchonine salts; many of the cinchona alkaloids undergo a similar rearrangement to toxines, so called because of their poisonous properties. The isomerism is due to the following change, the so-called "hydramine fission": [82]

$$\text{R—CHOH—CH—N—} \rightarrow \text{R—CO—CH}_2\ \text{NH—}$$
Cinchonine   Cinchotoxine
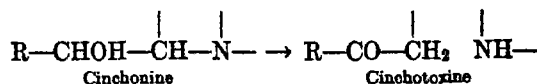
The cinchonatoxines can be converted through the cinchona ketones back to the alkaloids, a fact of great importance for synthesis (p. 1207).

Quinine, $C_{20}H_{24}O_2N_2$, is the most important of the cinchona alkaloids because of its extensive use as a febrifuge and antimalarial. Like

[81] Rabe, *Ann.*, **365**, 353 (1909); *Ber.*, **41**, 62 (1908).
[82] Rabe and Schneider, *Ann.*, **365**, 377 (1909).

cinchonine, quinine contains an alcoholic hydroxyl and a vinyl group, but possesses in addition a methoxyl group. By demethylation with hydrochloric acid it is split to methyl chloride and apoquinine, a phenolic base isomeric with the alkaloid cupreine. In the demethylation process a rearrangement takes place; methylation of apoquinine results in β-iso-quinine, an isomer of quinine.[63] The position of the quinine methoxyl group is evident from the appearance of 6-methoxyquinoline-4-carboxylic acid (quininic acid) in oxidations of quinine.
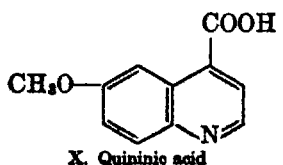
X. Quininic acid

The other products from the oxidation, namely meroquinene, cincholoiponic acid, and loiponic acid, show that the quinuclidine portion is the same as in cinchonine; quinine is 6'-methoxycinchonine.

A total synthesis of the complex structure present in the cinchona group has been accomplished by Rabe [64] in the preparation of the isomeric alkaloids hydroquinine and hydroquinidine. Both these bases are present in cinchona bark; hydroquinine is formed when quinine is hydrogenated. The quininic acid necessary for the synthesis was prepared by condensation of p-anisidine with acetoacetic ester, followed by ring closure, elimination of the phenolic hydroxyl, and oxidation.
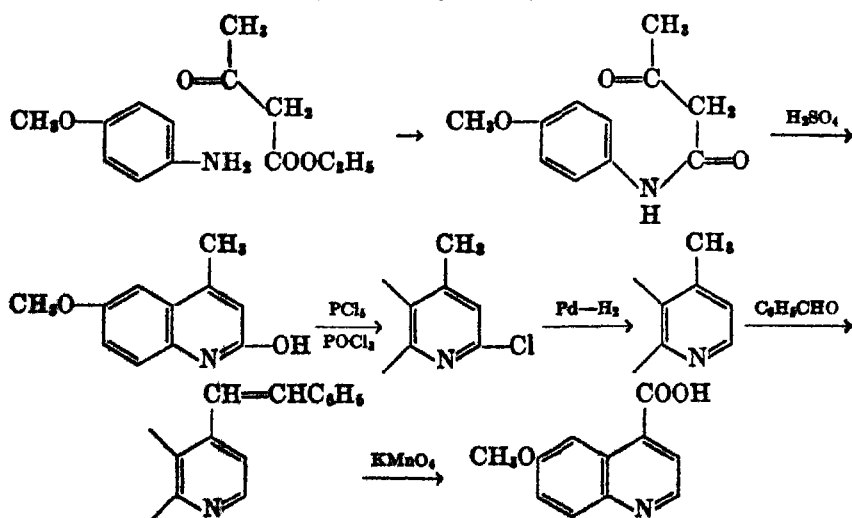
[63] Hesse, *Ann.*, **205**, 322 (1880); *Ber.*, **28**, 1301 (1895); Jarzyński, Ludwicsakówna, and Sussko, *Rec. trav. chim.*, **52**, 839 (1933).

[64] Rabe, Huntenburg, Schultse, and Voiger, *Ber.*, **64**, 2487 (1931).

The second necessary constituent, homocincholoipon, was prepared from 3-ethyl-4-methylpyridine (β-collidine). β-Collidine was condensed with chloral and the product converted to ethylpyridylacrylic acid with sodium ethoxide. On hydrogenation, the ethylpyridylacrylic acid gave a mixture of four optically isomeric ethylpiperidylpropionic acids. Resolution of the ethyl esters of these acids with tartaric acid yielded in large part the desired homocincholoipon.

N-Benzoylhomocincholoipon ethyl ester was condensed in the presence of sodium ethoxide with quininic ethyl ester to hydroquinotoxine, and the toxine transformed by bromination into hydroquininone, which on hydrogenation gave, according to the conditions, hydroquinidine or the stereoisomeric hydroquinine, identical with the natural bases.
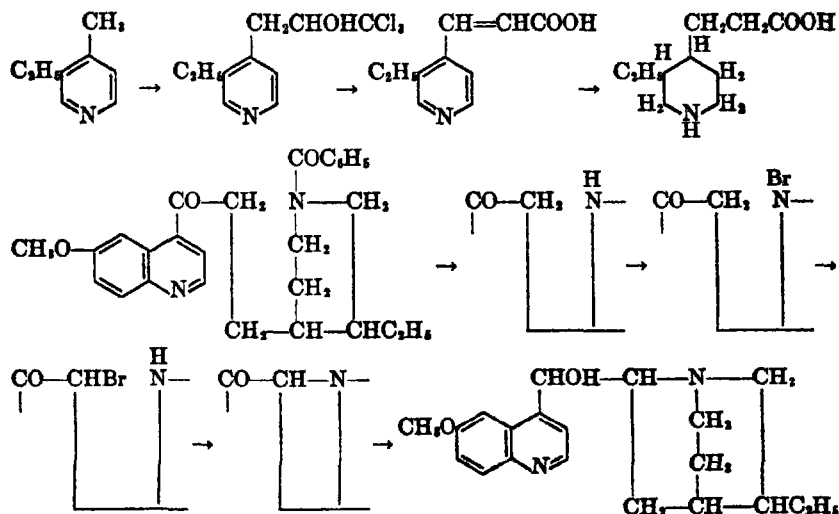
Extensive studies by Rabe [35] on the stereochemistry (p. 335) of the numerous isomers in the cinchona series have shown that, in the sixteen cinchona alcohols investigated, the steric arrangement of asymmetric atoms 3 and 4 is the same. Each of the pairs of isomers, cinchonine and
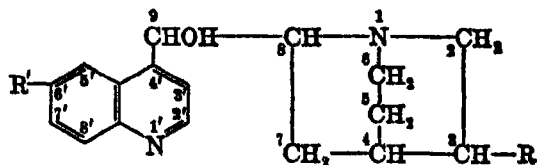
cinchonidine (R' = H, R = CH=CH₂), hydrocinchonine and hydrocinchonidine (R' = H, R = C₂H₅), quinine and quinidine (R' = OCH₃,

⁸⁵ Rabe, Ber., 55, 522 (1922); Ann., 492, 242 (1932).

R = CH=CH$_2$), hydroquinine and hydroquinidine (R' = OCH$_3$, R = C$_2$H$_5$), yields one ketone on oxidation. This fact, however, does not supply valid proof that the isomerism depends upon the configuration at C-9 alone, since the ketones exist in solution as an equilibrium mixture of two keto and two enol forms.
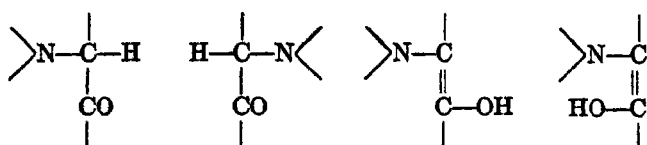
Reduction of the ketone regenerates the two alkaloids together with two new epimeric alcohols. On conversion of hydrocinchoninone to the desoxy derivative (at C-9, CO → CH$_2$), two stereoisomeric products are obtained, whence the conclusion can be drawn that both C-8 and C-9 are involved in the isomerism of the pairs named above. The two new alcohols obtained from reduction of a given cinchona ketone complete the number of stereoisomers (four) to be expected from configurational differences at the asymmetric centers C-8 and C-9.

Cupreine, C$_{19}$H$_{22}$O$_2$N$_2$, is found in the form of a molecular compound with quinine in the bark of *Remijia* species. It takes its name from the blue color of the bark. The structure of cupreine (R' = OH, R = CH=CH$_2$) is evident from the fact that it is phenolic in nature, and on methylation is converted to quinine. The ethyl ether of dihydrocupreine (R' = OC$_2$H$_5$, R = C$_2$H$_5$), a homolog of dihydroquinine, is an effective agent for the treatment of pneumococcus infections, and is used as the hydrochloride under the name "Optochin."

The cinchona alkaloids are marked by a toxic action on protoplasm, especially on low organisms, specifically on malaria parasites. Quinine is especially effective, and has in addition an antipyretic effect resulting from direct action on the heat-producing foci.

Angostura Alkaloids. The bark of *Galipea cusparia* (*Galipea officinalis*, angostura bark), which is employed in the West Indies as a febrifuge and finds further extensive use in bitter flavoring extracts, contains a variety of quinoline bases. The structures of some of these, cusparine, galipine, galipoline, and 2-*n*-amyl-4-methoxyquinoline, have been elucidated by E. Späth;[36] the nature of cuspareine and galipoidine is still uncertain.

Galipine, C$_{20}$H$_{21}$O$_3$N, and cusparine, C$_{19}$H$_{17}$O$_3$N, both yield protocatechuic acid when fused with alkali; this fact, together with the results

[36] Späth and co-workers, *Ber.*, **57**, 1243, 1687 (1924); *Monatsh.*, **52**, 129 (1929); **55**, 352 (1930).

of the methoxyl determination and the relationship of the empirical formulas, suggests that the second differs from the first only in containing a methylenedioxyl group in place of two methoxyl groups. Controlled oxidation of galipine results in 4-methoxyquinoline-2-carboxylic acid and veratric acid.

OCH₃

4-Methoxyquinoline-2-carboxylic acid

Veratric acid
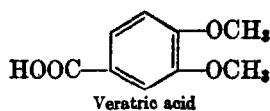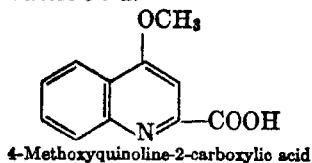
The formula derived for galipine by linkage of these two fragments through a C_2H_4 chain was shown to be correct by synthesis. 4-Methoxy-2-methylquinoline was condensed with veratraldehyde in the presence of zinc chloride, and the unsaturated product hydrogenated; the resulting base was identical with natural galipine. By a parallel reaction with piperonaldehyde, cusparine was obtained.

Galipine

Cusparine
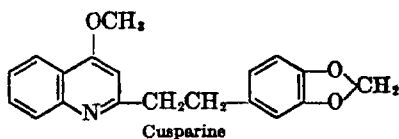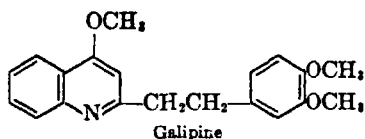
Galipoline is a phenolic base, differing from galipine in its formula by CH_2. On methylation it is converted to galipine. A choice between the three possible formulas was made by synthesis, and galipoline was shown to be a galipine demethylated at the 4-position of the quinoline nucleus. According to Schöpf, these quinoline bases are probably formed in the plant through condensation of o-aminobenzaldehyde with various β-keto acids (p. 1254).

ISOQUINOLINE GROUP. MESCAL, HYDRASTIS, BERBERIS, AND OPIUM ALKALOIDS

Alkaloids containing the isoquinoline (or tetrahydroisoquinoline) nucleus are scattered through a number of plant families, the *Cactaceae, Papaveraceae, Ranunculaceae, Menispermaceae,* and others. Associated with them in a few cases are open-chain bases whose relationship to the cyclic alkaloids is close.

Mescal Alkaloids. The flowering heads of several species of *Anhalonium* or *Lophophora* cactus, known as mescal buttons, have long been used as an intoxicant ("pellote," "peyotl") by the natives of Mexico and the southwestern portion of the United States. Dried slices of

the plant are chewed as a part of primitive ceremonial rites, and aqueous or alcoholic extracts from the buttons are also consumed for their exhilarating effect. The excitement and the color and sound hallucinations experienced probably arise largely from the action of mescaline.

Mescaline is oxidized by potassium permanganate to trimethylgallic acid; its general behavior shows further that the basic portion of the molecule is not cyclic in nature. The structural formula rests on the syntheses of Späth [87] and others.[88] Anhaline likewise has its nitrogen atom in an open chain, and is identical with hordenine, a base found in barley germs.
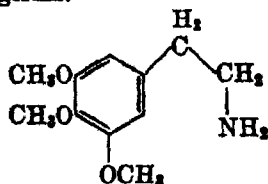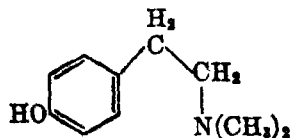
I. Mescaline   II Anhaline (Hordenine)

The remaining members of the anhalonium group, anhalamine, anhalonidine, anhalonine, pellotine, lophophorine, anhalinine, and anhalidine, are tetrahydroisoquinoline types.
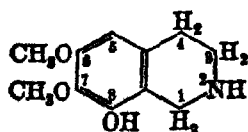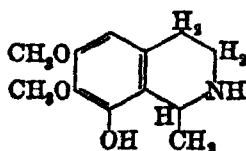
III. Anhalamine   IV. Anhalonidine   V. Anhalonine
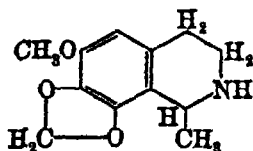
Pellotine and lophophorine are the N-methyl derivatives of anhalonidine and anhalonine respectively. Anhalinine represents the O-methyl derivative of anhalamine; anhalidine is the N-methyl derivative of anhalamine. The structure of the entire series has been demonstrated by synthesis.[89] The synthetic methods for the individual members of the group vary, but the synthesis of anhalamine may serve as an example. 3,4-Dimethoxy-5-benzyloxybenzaldehyde (VI) was condensed with nitromethane, and the ω-nitrostyrene derivative (VII) so obtained was reduced to the corresponding β-phenylethylamine derivative (VIII). Condensation of the phenylethylamine with formaldehyde resulted in closure of the tetrahydroisoquinoline ring (IX), and on removal of the benzyl group by hydrolysis anhalamine was obtained.

[87] Späth, Monatsh., 40, 129 (1919).
[88] Slotta and Heller, Ber., 63, 3029 (1930); Kindler and Peschke, Arch. Pharm., 270, 410 (1932); Hahn and Wassmuth, Ber., 67, 696 (1934).
[89] Späth and Becke, Monatsh., 66, 327 (1935).
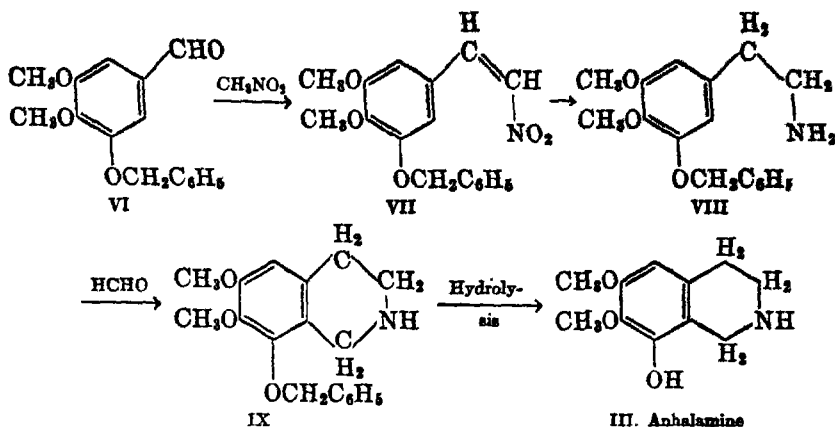
The tetrahydroisoquinoline ring closure, VIII to IX, might take place either *ortho* or *para* to the benzyloxyl group. A decision in favor of the *ortho*-position was reached by degradation of anhalamine ethyl ether, which yielded the anhydride X, instead of XI, which must have resulted from the alternative possibility.
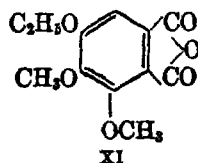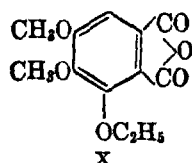
Hydrastis Alkaloids. The rhizomes of *Hydrastis canadensis* (golden seal) contain the three alkaloids hydrastine, berberine, and canadine, of which hydrastine, $C_{21}H_{21}O_6N$, is the most important. It is closely related to the opium alkaloid narcotine, which is 8-methoxyhydrastine; the two bases present a complete analogy in their reactions.

Hydrastine is a tertiary base carrying a methyl group on nitrogen.
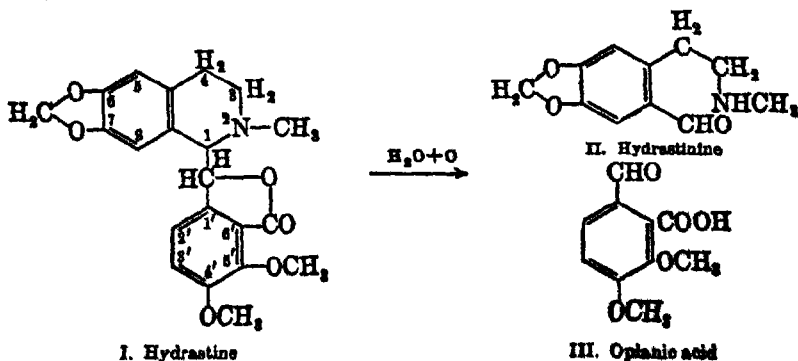
Two methoxyl groups and a methylenedioxyl group are present; the remaining two oxygen atoms are found in a lactone linkage.

The hydrastine structural formula was developed largely through the researches of Freund and of E. Schmidt. On oxidative hydrolysis, the molecule is split into hydrastinine and opianic acid; [90] the structure of the latter is known from relationships to methylvanillin (decarboxylation) and to hemipinic acid (oxidation). On heating alone, hydrastine yields as the non-basic part meconin, the lactone of meconinic acid.

IV. Methylvanillin   V. Opianic acid   VI. Hemipinic acid   VII. Meconin
(Veratraldehyde)
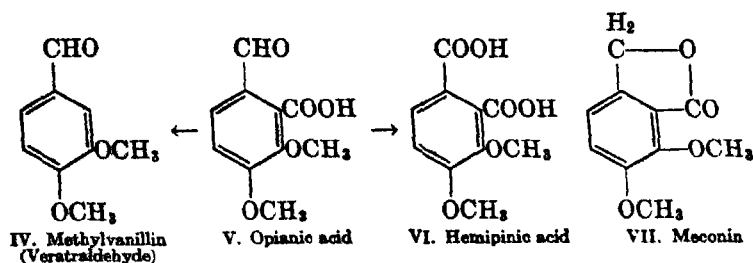
The basic portion from the above oxidative hydrolysis, hydrastinine, behaves as an aldehyde and as a secondary amine. Numerous reactions, as well as evidence from absorption spectra, indicate that hydrastinine (like its analog cotarnine) exists in three tautomeric forms:

Imino aldehyde   Pseudo-base
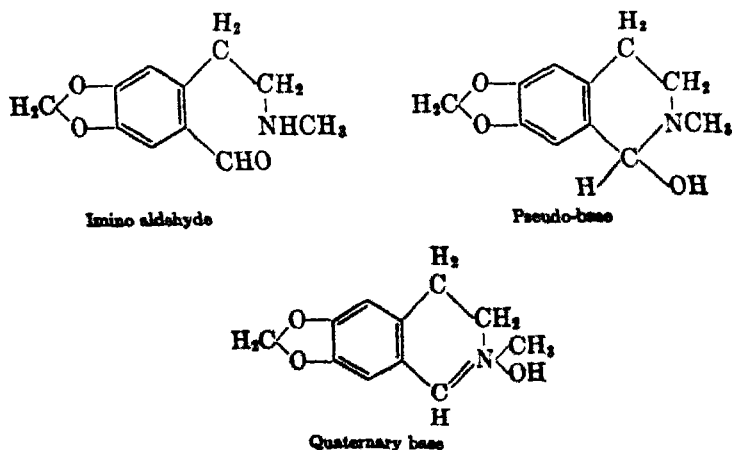
Quaternary base

Salt formation takes place through the quaternary ammonium form with loss of a molecule of water (Formula XII). Reduction of hydrastinine salts results in hydrohydrastinine (XV), an N-methyltetrahydroisoquinoline derivative.

[90] Freund and Will, Ber., 20, 88 (1887).

Exhaustive methylation of hydrastinine gives the nitrogen-free aldehyde, hydrastal (VIII). The constitution of hydrastal rests upon oxidation to hydrastic acid (IX), the methylene ether of 4,5-dihydroxyphthalic acid. Hydrastic acid is known as the end product from the degradation of many natural substances, and has been synthesized in several ways.
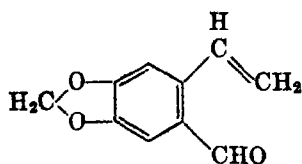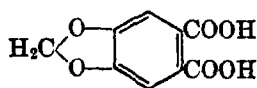
VIII. Hydrastal   IX. Hydrastic acid

The therapeutic value of hydrastinine in reducing uterine hemorrhage has led to the development of practicable syntheses [91] through modification of Decker's method.[92] In this synthesis, formylhomopiperonylamine is subjected to a Bischler-Napieralski reaction,[93] a cyclodehydration accomplished in the presence of phosphorus pentachloride, and the resulting norhydrastinine is converted to hydrastinine salts by the action of methyl halides.
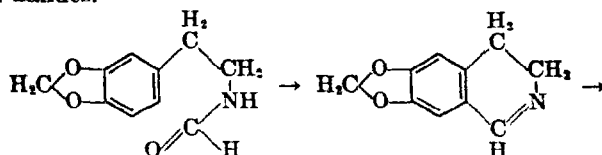
X. Formylhomopiperonylamine XI. Norhydrastinine
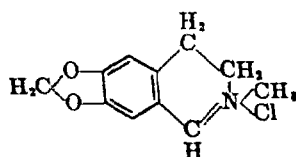
XII Hydrastinine chloride

Hydrastinine can also be prepared from the less valuable cotarnine (8-methoxyhydrastinine), a degradation product of narcotine. Cotarnine is reduced in acid medium to hydrocotarnine (XIV) (p. 1220), which, on further reduction with sodium and alcohol, suffers replacement of the methoxyl group by hydrogen.[94] The resulting hydrohydrastinine (XV) is converted by oxidation into hydrastinine.

[91] Rosenmund, Ber. deut. pharm. Ges., 29, 200 (1919); Kindler and co-workers, Ann., 431, 228 (1923); Arch. Pharm., 265, 389 (1927); 270, 353 (1932).

[92] Decker and co-workers, Ann., 395, 299, 321, 328 (1913).

[93] Bischler and Napieralski, Ber., 26, 1903 (1893).

[94] Pyman and Remfry, J. Chem. Soc., 101, 1595 (1912). This unusual type of reaction has been observed with a number of derivatives of pyrogallol trimethyl ether.

XIII. Cotarnine chloride   XIV. Hydrocotarnine

XV. Hydrohydrastinine   XVI. Hydrastinine salt
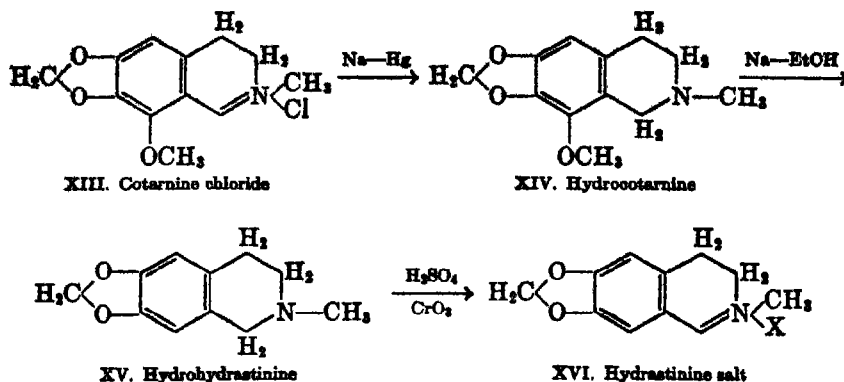
Linkage of the meconin and hydrastinine nuclei as in formula I is assumed in analogy with the structure demonstrated for narcotine (p. 1220). Attempts to synthesize d,l-hydrastine by a method successfully employed in the preparation of d,l-narcotine have resulted in two inactive hydrastine isomers, whose relation to natural l-hydrastine is not known.[95]

The alkaloid berberine, $C_{20}H_{19}O_5N$, is found not only in *Hydrastis*, but also in a number of unrelated plant families, notably the *Berberidaceae*, from which it takes its name. The structural formula has been developed largely through the researches of W. H. Perkin, jun.[96]

Berberine, like hydrastinine and cotarnine, forms its salts with loss of a molecule of water and, like these bases, behaves as though it existed in three forms:

XVII. Imino aldehyde   XVIIa. Pseudo-base   XVIIb. Quaternary base
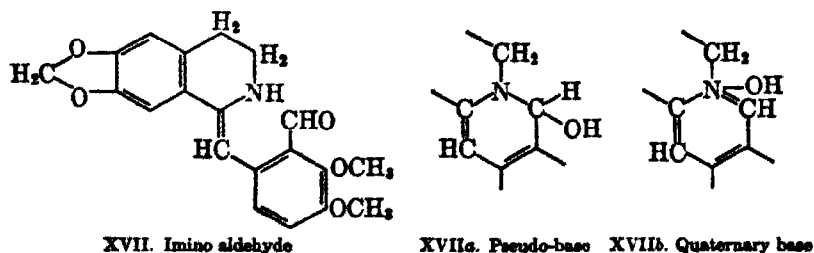
Information concerning the structure of berberine has been obtained largely through oxidation. With permanganate, hemipinic acid, hydrastic acid, oxyberberine, and berberal are obtained; with nitric acid, berberonic acid results.

[95] Hope, Pyman, Remfry, and Robinson, *ibid.*, 236 (1931).

[96] Perkin, jun., *ibid.*, **55**, 63 (1889); **57**, 992 (1890); Perkin and Robinson, *ibid.*, **97**, 305 (1910); Perkin, *ibid.*, **113**, 492, 722 (1918).

COOH

HOOC―OCH₃
OCH₃

VI. Hemipinic acid

H₂C(O―O)COOH
COOH

IX. Hydrastic acid

HOOC―N
COOH
COOH
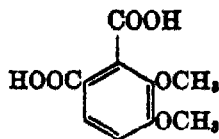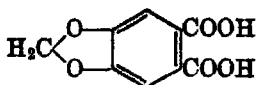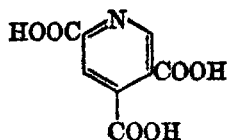
XVIII. Berberonic acid

The construction of a reasonable berberine formula from these smaller fragments was accomplished through a study of berberal. This base breaks down in hydrolytic processes into noroxyhydrastinine and pseudoopianic acid; these two components can also be united with loss of water to give berberal. Pseudoöpianic acid is an isomer of the opianic and metaopianic acids arising from the degradation of narcotine and cryptopine, respectively; in combining with noroxyhydrastinine it is assumed to react in the hydroxyphthalide form (XXI), and the product, berberal, is given the structure XIX. The structure of noroxyhydrastinine was shown by the relationship to the N-methyl derivative oxyhydrastinine, a substance obtained from hydrastinine by Cannizzaro's reaction.

From a consideration of berberal and of the reactions of berberine, Perkin, Gadamer,[97] and Faltis [98] advanced the now accepted formula of berberine, which has been substantiated by several syntheses.[99] The

XIX. Berberal

+H₂O
―H₂O

XX. Noroxyhydrastinine

XXI. Pseudoöpianic acid
(Hydroxyphthalide form)
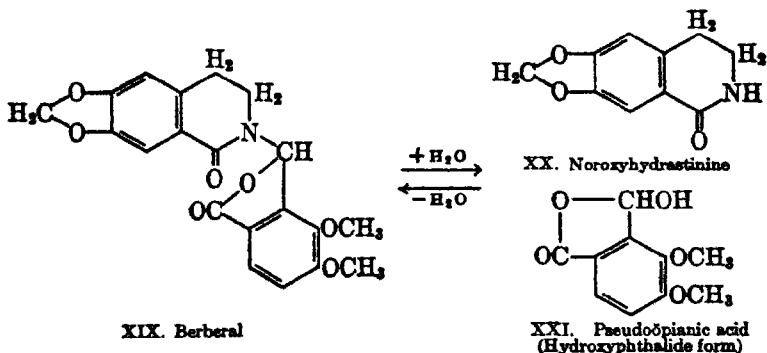
synthetic methods have in general as their goal oxyberberine (XXV), a base that has been obtained by gentle oxidation of berberine, or that is formed along with hydroberberine by a Cannizzaro intermolecular oxidation-reduction reaction when berberine is heated with alkali. In

[97] Gadamer, Arch. Pharm., 239, 648 (1901).

[98] Faltis, Monatsh., 31, 557 (1910).

[99] Pictet and Gams, Compt. rend., 152, 1102 (1911); 153, 386 (1911); Ber., 44, 2036, 2480 (1911); Perkin, J. Chem. Soc., 113, 737 (1918); Haworth, Perkin, and Rankin, ibid., 125, 1686 (1924); Perkin, Ray, and Robinson, ibid., 127, 740 (1925); Späth and Quietensky, Ber., 58, 2267 (1925).

this change, berberine must be considered as reacting in the pseudo-base form XVII*a*. Oxyberberine can be reduced to tetrahydroberberine, and the latter converted to berberine by oxidation. The oxyberberine synthesis of Perkin, Rây, and Robinson consisted in condensation of homopiperonylamine and meconincarboxylic acid to an amide (XXIV), which was then subjected to a Bischler-Napieralski isoquinoline ring closure. This resulted in an intermediate, which was reducible to oxyberberine.

XXII. Homopiperonylamine

XXIII. Meconincarboxylic acid   XXIV. Meconincarboxyhomo-   XXV. Oxyberberine
                                        piperonylamide
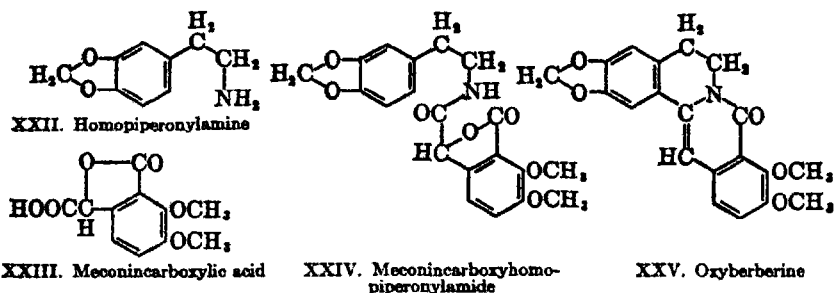
Berberine is relatively inactive physiologically; in large doses it exerts a paralyzing effect of central origin. It has been variously recommended as an oxytocic, as an antimalarial, and as a cure for morphinism.

Canadine is *l*-tetrahydroberberine,[97, 100] and occurs also as the dextro form in *Corydalis* [101] along with other alkaloids (corydaline, corybulbine, etc.) closely related to berberine.

Opium Alkaloids.[102] Opium is the dried latex from the unripe seed capsules of the opium poppy, *Papaver somniferum*, and has proved to be one of the richest sources of alkaloids. The bases occur in part in the free state, in part combined with sulfuric, lactic, acetic, or meconic acids. Most of them are derivatives of isoquinoline or tetrahydroisoquinoline; the remainder (morphine group) are probably related phytochemically to those of the isoquinoline group,[1, 2, 3] and are most conveniently considered under this classification.

Because of the great importance of opium and its alkaloids in medicine, the world production is enormous. In 1939, 1,123,164 kg. of raw opium were reported to the League of Nations, and in the same year the manufacture of 27,238 kg. of morphine alkaloid was recorded. These figures, however, do not include the huge quantities produced illegitimately to supply the needs of opium and morphine addicts. The clandestine production probably exceeds 5000 tons of opium.

[100] Gadamer, *Arch. Pharm.*, **248**, 43 (1910).

[101] Spāth and Julian, *Ber.*, **64**, 1131 (1931).

[102] Small and Lutz, "Chemistry of the Opium Alkaloids," U. S. Government Printing Office (1932); Kappelmeier, "Die Konstitutionserforschung der wichtigsten Opium Alkaloide," *Ahrens' Samml. chem. chem.-tech. Vorträge*, Vol. 18, p. 225 (1912).

Papaverine, $C_{20}H_{21}O_4N$, is found in all parts of the growing poppy, and is present in opium to the extent of about 0.5 to 1 per cent. Its structure as tetramethoxy-1-benzylisoquinoline was elucidated in a long series of researches by Goldschmiedt.[103] These investigations constitute an excellent example of the application of oxidative degradation to structure determination. By gentle oxidation of papaverine, the secondary alcohol papaverinol is formed;[104] more vigorous treatment yields the cor-
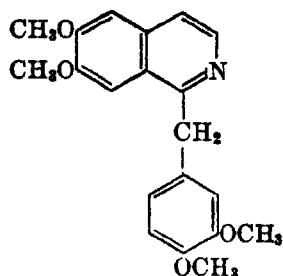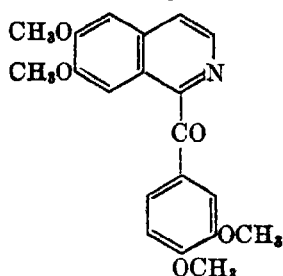
I. Papaverine   II. Papaveraldine   III. Papaverinic acid
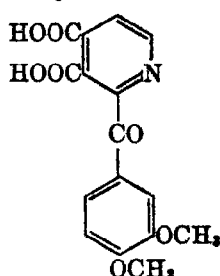
responding ketone, papaveraldine,[105] or finally the dibasic acid, papaverinic acid.[106]

On more complete oxidation, the fragments obtained are veratric acid, metahemipinic acid, 2,3,4-pyridinetricarboxylic acid, and 6,7-dimethoxyisoquinoline-1-carboxylic acid.[106]
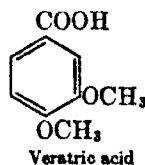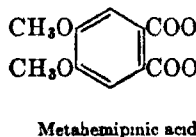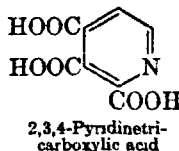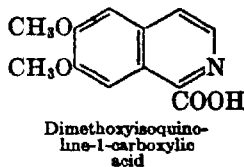
Veratric acid   Metahemipinic acid   2,3,4-Pyridinetri-carboxylic acid   Dimethoxyisoquino-line-1-carboxylic acid

On fusion with potassium hydroxide, papaverine yields, among other products, 6,7-dimethoxyisoquinoline and dimethylhomocatechol.[107]
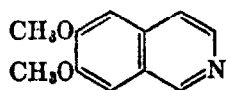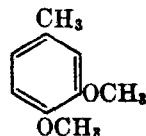
6,7-Dimethoxyisoquinoline   Dimethylhomocatechol

The mode of union of these fragments in papaverine is evident: the appearance of two methoxyl groups in each portion, as well as in the oxidation products above, shows that the methoxyls do not take part in

[103] Goldschmiedt, Monatsh., 9, 778 (1888).
[104] Gadamer and Schulemann, Arch. Pharm., 253, 284 (1915).
[105] Goldschmiedt, Monatsh., 6, 954 (1885); 7, 485 (1886).
[106] Goldschmiedt, ibid., 6, 372 (1885); Goldschmiedt and Strache, ibid., 10, 692 (1889).
[107] Goldschmiedt, ibid., 8, 510 (1887).

the linkage; a direct union of two aromatic nuclei would not explain the ease with which they separate. Linkage through a methylene group at the point where the carboxyl of dimethoxyisoquinoline-1-carboxylic acid is found gives a satisfactory explanation of these facts and of the other reactions of papaverine.

Papaverine was first synthesized by Pictet and Gams [109] by a method that gave complete confirmation to the accepted structure. As starting substances for this synthesis, veratrole (o-dimethoxybenzene) and veratric acid (3,4-dimethoxybenzoic acid) were chosen. By the Friedel and Crafts reaction veratrole was converted to acetoveratrone, and the isonitroso derivative of acetoveratrone was reduced with tin and hydrochloric acid to aminoacetoveratrone (IV). Interaction of aminoacetoveratrone hydrochloride and homoveratroyl chloride (V) yielded the amide, ω-(homoveratroylamido)acetoveratrone (VI). By selective reduction of the ketonic carbonyl group of VI with sodium amalgam, the corresponding secondary alcohol, homoveratroylhydroxyhomoveratrylamine, was obtained. This substance, heated in xylene with phosphorus pentoxide, lost two molecules of water, closing the isoquinoline ring to give papaverine.

IV   V   VI

VII   I
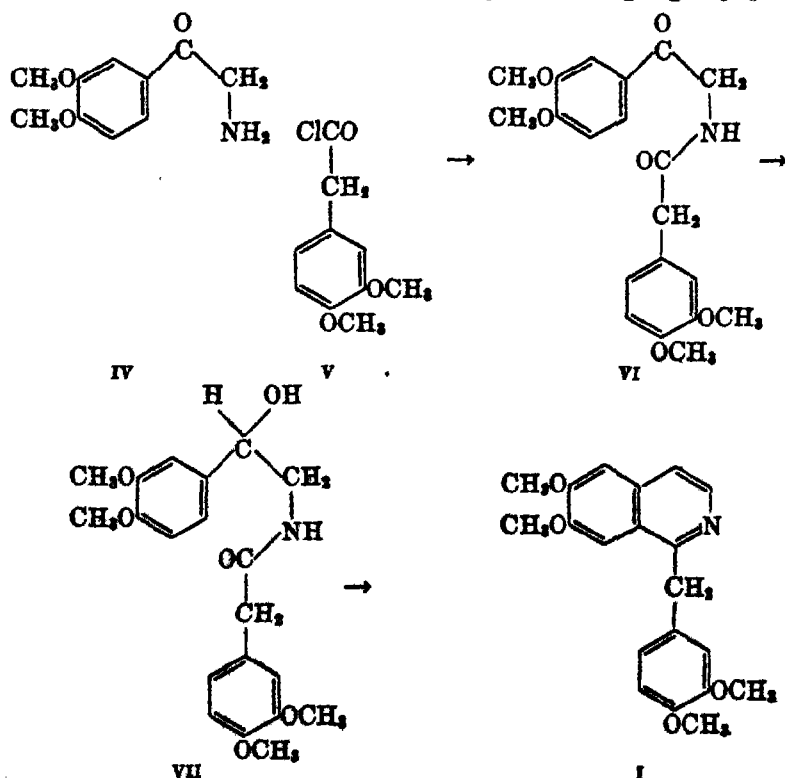
[109] Pictet and Gams, *Ber.*, **42**, 2943 (1909).

Numerous other syntheses have been developed; [109] it is reported that secret processes developed by drug manufacturers permit the synthesis of papaverine on any desired scale.

Papaverine causes light narcosis, in larger doses tetanus and respiratory paralysis. It has an antispasmodic action on smooth muscle and is used (chiefly in Europe) to relieve bronchial or intestinal spasms, and in obstetrics.

Laudanosine, $C_{21}H_{27}O_4N$, is found in opium in small amounts, and is closely related to papaverine. Its structure as *dextro*-tetrahydro-N-methylpapaverine was demonstrated by reduction of papaverine methochloride with tin and acid, and resolution of the resulting *d,l*-tetrahydro-N-methylpapaverine (racemic laudanosine); the *dextro* form was identical with laudanosine.[110] The first complete synthesis of laudanosine was carried out by Pictet and Finkelstein [111] and is of interest as the first synthesis of an opium alkaloid. In connection with laudanosine, the rare opium alkaloids laudanine, laudanidine, and codamine may be mentioned. Laudanine is the racemic form of 3'-demethylo-tetrahydro-N-methylpapaverine; [112] laudanidine is the *levo* form of the same base.[113] Codamine represents racemic 7-demethylo-tetrahydro-N-methylpapaverine.[114]

Laudanosine   Laudanine   Codamine
                     Laudanidine
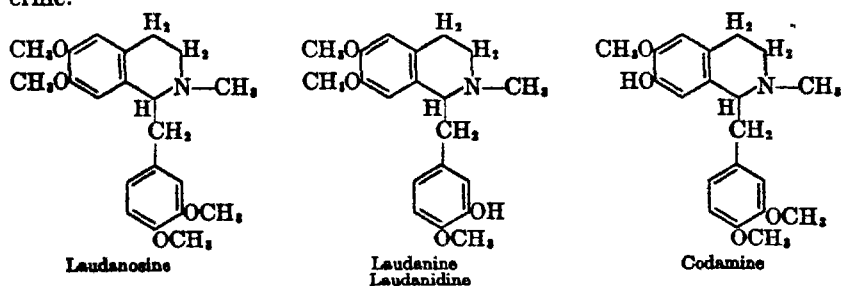
The location of the phenolic hydroxyl groups in laudanine and codamine was shown by Späth through the device of ethylation and subsequent oxidation. From ethyllaudanine, 3-ethoxy-4-methoxybenzoic acid was obtained, from ethylcodamine, an isoquinoline derivative carrying the ethoxyl group in position 7; the structure of both alkaloids was then confirmed by synthesis.

[109] Buck, Haworth, and Perkin, jun., *J. Chem. Soc.*, **125**, 2176 (1924); Rosenmund, Nothnagel, and Riesenfeldt, *Ber.*, **60**, 392 (1927); Späth and Burger, *Ber.*, **60**, 704 (1927); Buck, *J. Am. Chem. Soc.*, **52**, 3610 (1930); Späth and Berger, *Ber.*, **63**, 2098 (1930); Mannich and Walther, *Arch. Pharm.*, **265**, 1 (1927).

[110] Pictet and Athanasescu, *Ber.*, **33**, 2346 (1900).

[111] Pictet and Finkelstein, *Ber.*, **42**, 1979 (1909).

[112] Späth and Lang, *Monatsh.*, **42**, 273 (1921).

[113] Späth and Bernhauer, *Ber.*, **58**, 200 (1925); Späth and Burger, *Monatsh.*, **47**, 733 (1926).

[114] Späth and Epstein, *Ber.*, **59**, 2791 (1926); **61**, 334 (1928).

The alkaloid narcotine, $C_{22}H_{23}O_7N$, occurs in opium as the free base in amounts up to 10 per cent or more. It differs structurally from hydrastine (p. 1211) only by the presence of a methoxyl group in the 8-position. On oxidative hydrolysis it is broken down to cotarnine (the methoxy analog of hydrastinine) and opianic acid.[115, 116] From reductive hydrolysis, the fragments are meconin and the previously known opium alkaloid hydrocotarnine.[116]

Narcotine

Hydrocotarnine

Meconin
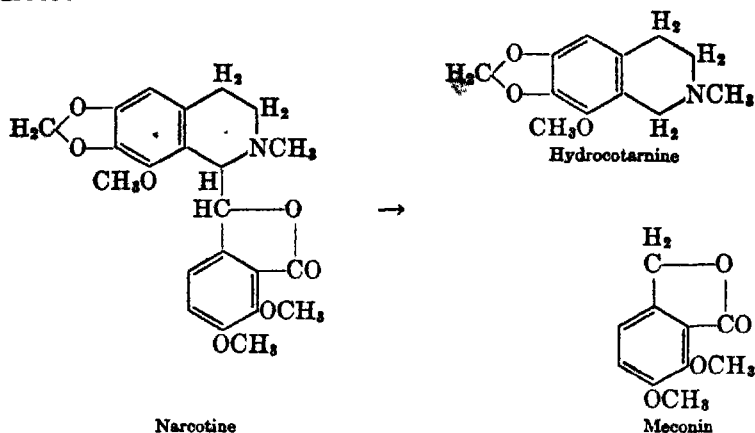
Cotarnine presents in its reactions and tautomeric behavior a complete analogy to hydrastinine. On reduction it yields hydrocotarnine, the analog of hydrohydrastinine; on oxidation the product is cotarnic acid (methoxyhydrastic acid). When treated with bromine, cotarnine is converted to a series of hydroxyisoquinoline betaines known as tarconines.[102]

The structural formula of narcotine, like that of hydrastine, was evolved by joining in the most reasonable manner the products identified from degradation. The presence of a tertiary nitrogen atom shows that the nitrogen-containing portion in narcotine has the isoquinoline structure of hydrocotarnine and not the open-chain amine (or tautomeric) form of cotarnine. The lactone nature of narcotine indicates the meconin, rather than the opianic acid, grouping for the nitrogen-free portion, and the appearance of two aldehyde groups (in cotarnine and opianic acid) in oxidative degradation shows the points at which the two fragments are joined.[117] The structural concept so reached was confirmed by the synthesis of Perkin and Robinson.[118] Meconin and cotarnine (the

[115] Wöhler. Ann., 50, 1 (1844).

[116] Beckett and Wright, J. Chem. Soc., 28, 573 (1875); Rabe and McMillan, Ber., 43, 800 (1910).

[117] Roser, Ann., 254, 356 (1889).

[118] Perkin and Robinson, J. Chem. Soc., 99, 775 (1911).

latter probably reacting as the pseudo-base) were condensed, giving the opium alkaloid gnoscopine, which is *d,l*-narcotine; the *levo* form is natural narcotine. Since both of the constituents have been synthesized, this constitutes a complete narcotine synthesis.

The Morphine Alkaloids. Morphine was the first organic base to be isolated and characterized as such (Sertürner, 1805); [119] it is today one of the most useful drugs known. Opium may contain as much as 20 per cent morphine, but the average is in the neighborhood of 10 per cent. Smoking opium, a specially prepared form, has about 8 per cent morphine.[120] The methyl ether of morphine, known as codeine, and the third member of the morphine group, thebaine, are present to the extent of about 0.5 per cent in opium. It is not known with certainty whether morphine and codeine occur in any plant but *Papaver somniferum*; thebaine has been found in *Papaver orientale*.

No group of alkaloids has offered more stubborn resistance to solution of the structural problem; since 1889, when the first well-founded speculation appeared (Knorr [121]), no less than twenty structural formulas for morphine have been proposed by eminent workers in the field.[102] The most probable of these, advanced by Gulland and Robinson in 1925,[1,122] is based upon the enormous amount of experimental evidence that has been accumulated in the last four decades, and explains best the complicated and exceptional reactions of the morphine group.
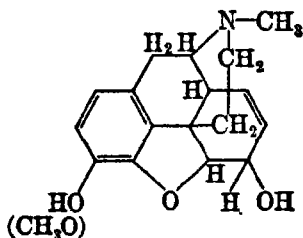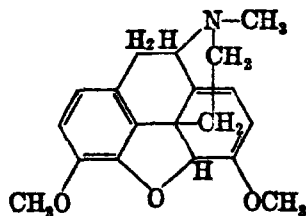
I. Morphine (Codeine)   II. Thebaine

Of the three oxygen atoms in morphine, $C_{17}H_{19}O_3N$, one is present in a phenolic hydroxyl, one in an alcoholic hydroxyl, and the third is indifferent, in an ether linkage. The nature of the last-named was shown by Vongerichten [123] through his studies on methylmorphenol. This substance is formed in the last step of the exhaustive methylation of morphine (or codeine) through a reaction peculiar to the morphine series.

[119] Krömeke, "Fr. Wilh. Sertürner, der Entdecker des Morphiums," Fischer, Jena (1925).

[120] Simons, *J. Ind. Eng. Chem.*, **8**, 345 (1916).

[121] Knorr, *Ber.*, **22**, 1113 (1889).

[122] Gulland and Robinson, *J. Chem. Soc.*, **123**, 980 (1923).

[123] Vongerichten, *Ber.*, **30**, 2439 (1897); **31**, 3198 (1898); **33**, 352 (1900).

Methylmorphenol is 3-methoxy-4,5-phenanthrylene oxide; it can be transformed to 3-methoxy-4-hydroxyphenanthrene (methylmorphol) by reduction with sodium and alcohol, or to 3,4,5-trihydroxyphenanthrene

III. Methylmorphenol   IV. Methylmorphol
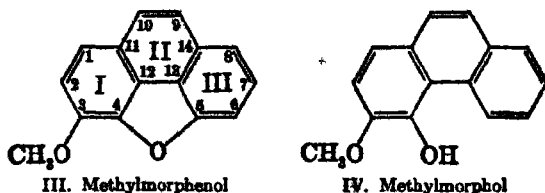
by alkali fusion. The location of two of the morphine oxygen atoms and the presence of the phenanthrene nucleus are thus demonstrated. By zinc dust distillation of morphine and its derivatives, phenanthrene itself is obtained.

Both hydroxyl groups of morphine are acetylated by acetic anhydride, yielding diacetylmorphine, whose hydrochloride is the important narcotic heroin. With chlorinating agents only the alcoholic hydroxyl group is attacked, and a phenolic halogenated base, α-chloromorphide, results; at the same time small amounts of an isomer, β-chloromorphide, are formed. The β-isomer represents a rearrangement product of the α-compound, and can be prepared from it; the nature of the isomerism is not certain, but it is probably due to attachment of the halogen at a different point in ring III, i.e., at carbon-8.

The two chloromorphides can be hydrolyzed to three isomers, known as α-, β-, and γ-isomorphine.[124] No morphine is regenerated in the hydrolysis. In nearly all morphine studies, structural determination has been made in the methyl ether (codeine) series because of the greater stability and more agreeable physical properties of these derivatives. In the reactions under consideration, codeine, through α- or β-chlorocodide, is converted to isocodeine, allopseudocodeine, and pseudocodeine, corresponding respectively to α-, β-, and γ-isomorphines.

Knorr[125] was able to show that codeine and isocodeine can be oxidized at the alcoholic hydroxyl group to give the same ketone, codeinone; the isomerism depends therefore only upon the spatial arrangement of hydrogen and hydroxyl in these two diastereoisomers. Codeinone, moreover, can be degraded to 3,4,6-trimethoxyphenanthrene. The alcoholic hydroxyl group in codeine and isocodeine, as well as in morphine and α-isomorphine, must be located on carbon-6. By a similar method, through pseudocodeinone and 3,4,8-trimethoxyphenanthrene it was shown that in allopseudo- and pseudo-codeines the hydroxyl group is on

[124] Lees, J. Chem. Soc., 91, 1408 (1907); Oppé, Ber., 41, 975 (1908).
[125] Knorr and Hörlein, Ber., 40, 2032, 3341, 4889 (1907); Knorr, Ber., 36, 3074 (1903).

carbon-8. The nuclear positions -6 and -8 are thus excluded as possible points of attachment of the nitrogen-containing ring.
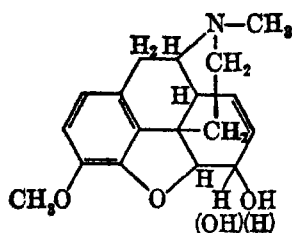
V. Codeine, Isocodeine
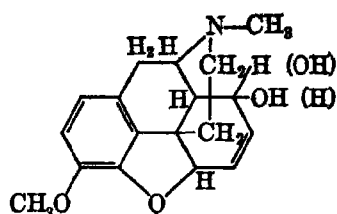
VI. Allopseudocodeine, Pseudocodeine
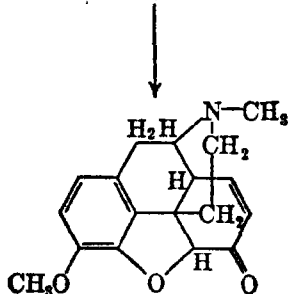
VII. Codeinone
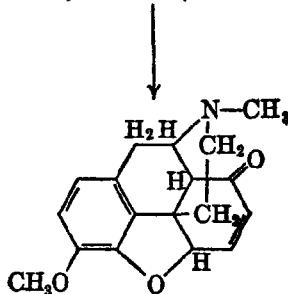
VIII. Pseudocodeinone

The presence of the alicyclic double bond in morphine and codeine and in the isomers can be demonstrated by catalytic hydrogenation; in pseudocodeine and allopseudocodeine a tendency to add four atoms of hydrogen with reductive scission of the 4,5-ether linkage is seen, a phenomenon that is undoubtedly connected with the allyl ether structure of these bases. The position assigned to the codeine double bond depends upon reactions of the methylmorphimethines.
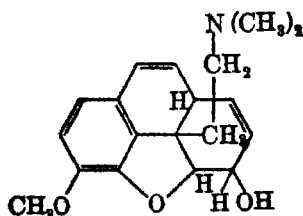
IX. α-Methylmorphimethine
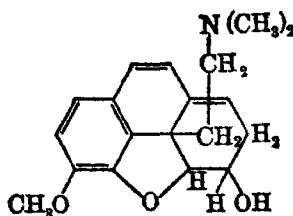
X. β-Methylmorphimethine

Satisfactory proof of the position of the nitrogen-containing ring in the morphine series has been most difficult to obtain. The nitrogen atom is tertiary and carries a methyl group. When codeine methiodide is heated with alkali, the nitrogen ring is broken in the usual way and the

product is α-methylmorphimethine.[126] Under the influence of alcoholic alkali, α-methylmorphimethine is transformed to an isomer, β-methylmorphimethine. The change is believed to be due to a shift of the 7,8-double bond to a position (-8,14) in conjugation with the new unsaturation at -9,10, and is one of the chief reasons for placing the morphine double linkage at position -7,8. These two methine bases yield the same tetrahydro derivative, showing that the isomerism is due only to a difference in position of the double bond. By the same degradation process, isocodeine gives γ-methylmorphimethine, which likewise can undergo rearrangement. From pseudocodeine and allo-pseudocodeine, however, methylmorphimethines (ε- and ζ-) are obtained in which the location of the unsaturation and hydroxyl is such as to preclude a shift to form a conjugated system.[126, 127]

When the methylmorphimethines are heated with acetic anhydride, they break down into methylmorphol (IV) and β-hydroxyethyldimethylamine, $(CH_3)_2NCH_2CH_2OH$. The nitrogen atom in the methines and in morphine is evidently linked with two carbon atoms in a chain which is easily separated as a whole from the phenanthrene nucleus. Degradation of the methylmorphimethine methiodides with alkali by the usual Hofmann procedure also results in loss of the chain in the form of trimethylamine and ethylene. The point of attachment of the nitrogen atom to the nucleus in morphine rests on Knorr's [128] studies of 9-hydroxy-codeine, a derivative obtained by gentle oxidation of codeine. The methylmorphimethine formed in the first step of the degradation of this hydroxycodeine is a ketone, hence the new hydroxyl group must be located on a carbon atom that becomes unsaturated when the nitrogen-containing ring opens.
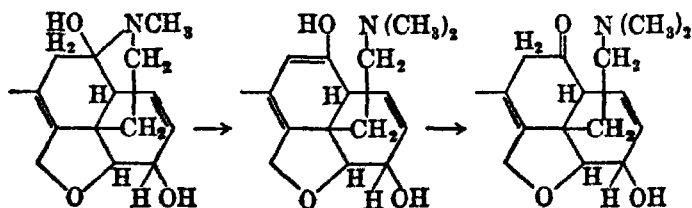
Only positions -9 or -10 are possible for the carbon atom in question, for acetolysis of the methine results in a methoxydiacetoxyphenanthrene that cannot be oxidized to a phenanthrene-9,10-quinone without loss of an acetoxyl group. On steric grounds, attachment at 9 is most probable.

[126] Hesse, *Ann.*, **222**, 203 (1883).

[127] Knorr and co-workers, *Ber.*, **35**, 3009, 3010 (1902); **39**, 4412 (1906); **40**, 3844 (1907); Schryver and Lees, *J. Chem. Soc.*, **79**, 563 (1901); Wieland and Koralek, *Ann.*, **433**, 267 (1923).

[128] Knorr and co-workers, *Ber.*, **39**, 1414 (1906); **40**, 2042 (1907).

Because of the great lability of the ethanamine chain, the location of its other end has been fraught with much difficulty. Treatment of morphine with various acidic reagents results in apomorphine, in which Pschorr demonstrated, both by degradation and by synthesis, that the chain is linked at position -8.[129] This position is untenable for morphine, however, because of the evidence cited above for the structure of pseudo-codeinone. Thebaine, which is known through its relationship to codeinone and to dihydromorphine dimethyl ether to contain the fundamental morphine skeleton, may give through the action of hydrochloric acid either morphothebaine (chain on carbon-8) or thebenine, in which the chain is unquestionably attached at carbon-5.[130]

XI. Apomorphine   XII. Morphothebaine

XIII. Thebenine
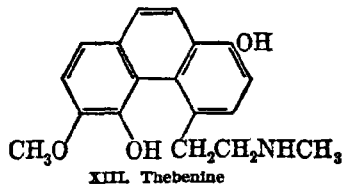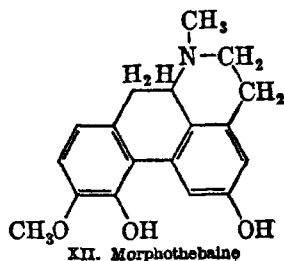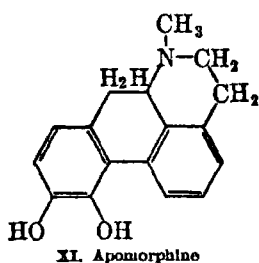
The generally accepted location of the chain at carbon-13 was developed in an attempt to account for the extraordinary tendency shown by all the members of the morphine group to lose the entire ethanamine side chain in degradative reactions. Linkage at a quaternary carbon atom (position -13 or -14) is the only arrangement under which it becomes necessary for the side chain to shift (to position -5 or -8) or separate from the molecule when aromatization of ring III or of the whole phenanthrene nucleus takes place. Position -14 is improbable because it does not permit of a reasonable structure for thebaine. Schöpf [2] was able to substantiate the structural theory of Gulland and Robinson by a study of the Beckmann rearrangement of dihydrocodeinone oxime (XIV), which resulted in formation of an aldehyde (XV), instead of the ketone that would be expected if the chain were attached in position -5.

[129] Pschorr and co-workers, Ber., 40, 1998 (1907); 62, 321 (1929); Späth and Hromatka, Ber., 62, 325 (1929).
[130] Gulland and Virden, J. Chem. Soc., 921 (1928).

XIV   XV
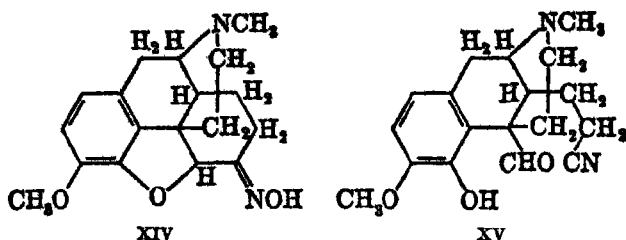
Thebaine (II) is regarded as the methyl ether of the enol form of codeinone (VII), and can be converted to codeinone by gentle hydrolysis. When the two hydroaromatic double bonds present in thebaine are saturated, dihydromorphine dimethyl ether is obtained.

In recent years, exhaustive researches have been carried out to determine the structural features responsible for the physiological action of morphine.[121] It may be stated briefly that the presence of the phenolic hydroxyl is essential for high analgesic action, while the alcoholic hydroxyl appears to exert an opposite effect. When the alcoholic hydroxyl group is replaced by hydrogen, or methylated, a great increase in analgesic power is observed. Morphine alcoholic methyl ether, for example, is approximately one hundred times as effective in this respect as the phenolic methyl ether (codeine). If the nitrogen- or oxygen-containing rings of morphine are opened, a great decrease in physiological action results.

Neopine, a recently discovered rare member of the morphine group, represents a codeine in which the alicyclic unsaturation lies between carbons-8 and -14. It is converted to dihydrocodeine on hydrogenation, or directly to β-methylmorphimethine in the first step of Hofmann's degradation.[122]

An alkaloid having a structural skeleton similar to that of the morphine group is found in the Japanese vine *Sinomenium acutum*.[123] This base, sinomenine, is a 7-methoxy derivative of the keto phenol thebainone in the morphine series.
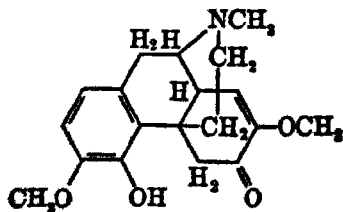
[121] Small, Eddy, Mosettig, and Himmelsbach, "Studies on Drug Addiction," U. S. Government Printing Office (1938).
[122] Van Duin, Robinson, and Smith, *J. Chem. Soc.*, 903 (1926).
[123] Kondo and Ochiai, *Ann.*, 470, 224 (1929).

All the asymmetric carbon atoms in sinomenine have the configuration opposite to that of the corresponding asymmetric centers in morphine; conversion of sinomenine to the optical antipodes of morphine derivatives has been accomplished.

Morphine finds therapeutic use as a result of its depressant action on different parts of the central nervous system. It causes marked analgesia and, in larger doses, narcosis. Codeine has more tendency to excite, and the narcotic effects of morphine are exhibited, but in weaker degree. Morphine and many of its derivatives are characterized by their ability to produce the dangerous addiction known as morphinism. Thebaine is a violent tetanic poison.

INDOLE GROUP. HYPAPHORINE, ABRINE, AND GRAMINE; HARMALA, PHYSOSTIGMINE, YOHIMBINE, STRYCHNOS, AND ERGOT ALKALOIDS

The important group of alkaloids containing the indole nucleus ranges in complexity from such simple substances as hypaphorine (I), the methylbetaine of tryptophan, to the complicated structures of yohimbine and strychnine. Probably all these alkaloids have as the parent substance the amino acid tryptophan (p. 1159), a building unit that appears to be of great importance in the synthesis of both plant and animal bases. The toad poison (p. 1164) bufotenine is a derivative of tryptamine; it is interesting to note that bufotenine and physostigmine (p. 1230) are the only 5-hydroxyindole derivatives that have been encountered in nature.[134]
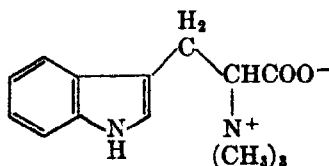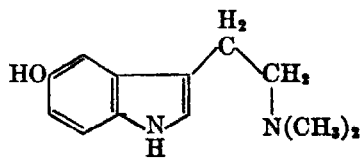
I. Hypaphorine   II. Bufotenine

Abrine, an alkaloid from the seeds of *Abrus precatorius*, is likewise a simple derivative of tryptophan. Its constitution as in formula III is immediately evident from the fact that it can be decarboxylated to yield N-methyltryptamine. The optical activity of abrine excludes a ring position for the carboxyl group. On treatment with methyl iodide and alkali, moreover, it gives the same methyl ester methiodide as is obtained from the parallel methylation of *l*-tryptophan.[135]

[134] Wieland, Kons, and Mittasch, *Ann.*, **513**, 1 (1934).
[135] Hoshino, *Ann.*, **520**, 31 (1935).
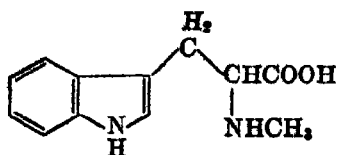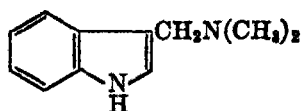
III. Abrine   IV. Gramine

Another simple indole base, gramine, has recently been isolated from the germ of Swedish barley. It is the first alkaloid to be found in any of the *Gramineae*,[136] and is identical with donaxine, an alkaloid obtained from an Asiatic reed. The presence of the indole nucleus in gramine or donaxine is apparent not only from the absorption spectrum, but also from the appearance of skatole (3-methylindole) in the zinc dust distillation. A synthesis of gramine has been reported.[137]

Harmala Alkaloids. The seeds of the African rue, *Peganum harmala*, contain as phosphates the alkaloids harmaline, $C_{13}H_{14}ON_2$; harmine, $C_{13}H_{12}ON_2$; and harmalol, $C_{12}H_{12}ON_2$. The three bases are closely related: harmaline is the methyl ether of harmalol and a dihydro derivative of harmine. The ring system present is a condensation of benzene, pyrrole, and pyridine nuclei, which Perkin and Robinson have designated as 4-carboline.
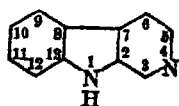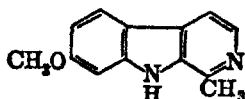
I. 4-Carboline   II. Harmine   III. Harmaline, Harmalol
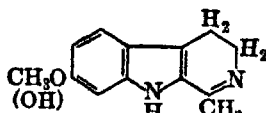
Oxidation of harmaline with nitric acid reveals the benzene nucleus, which appears as *m*-nitroanisic acid. In the same reaction, a dibasic acid, $C_{10}H_8O_4N_2$, harminic acid (VI), is formed. In it the pyrrole and pyridine nuclei are contained; on further oxidation it yields isonicotinic acid (γ-pyridinecarboxylic acid).
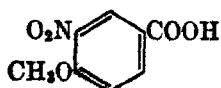
IV. *m*-Nitroanisic acid   V. Isonicotinic acid
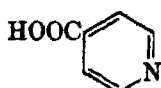
In harminic acid the two carboxyl groups derived from the benzene nucleus are adjacent (fluorescein reaction); by decarboxylation one

[136] von Euler and Erdtman, *Ann.*, **520**, 1 (1935); von Euler, Erdtman, and Hellström, *Ber.*, **69**, 743 (1936).

[137] Wieland and Hsing, *Ann.*, **526**, 188 (1936). See, also, Erdtman, *Ber.*, **69**, 2462 (1936); Orechoff and Norkina, *Ber.*, **68**, 436 (1935).

or both may be removed, giving respectively apoharminic acid or apoharmine.

HOOC
HOOC —N— =N
    H   CH₃
VI. Harminic acid

—N— =N
 H   CH₃
VII. Apoharmine
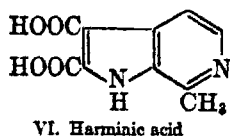
Further evidence for the presence of the pyrrole nucleus in harmaline is found in the formation of red dyestuffs through the action of diazonium salts. The location of the methyl group is deduced from the formation of benzylidene compounds by condensation with benzaldehyde, a reaction characteristic of α-methylpyridines; this leaves, however, two positions (3- and 5-) possible for the methyl group.

An important clue to the arrangement of the nuclei in the harmala alkaloids was obtained in the study of harman. This base, which is identical with the alkaloids arabine and loturine, was first prepared by demethoxylation of harmine; it was found to be genetically related to tryptophan, from which it can be obtained by oxidation with ferric chloride in the presence of alcohol.

H_2
C
CHCOOH
—N—   NH₂
 H
VIII Tryptophan

—N— =N
 H   CH₃
IX. Harman
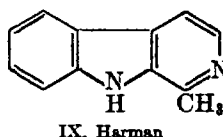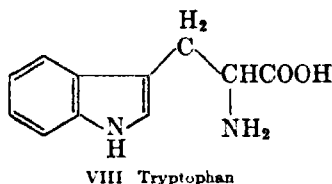
Perkin and Robinson [138] suggested that the phytochemical synthesis of harmaline proceeds through a condensation of decarboxylated hydroxy-tryptophan with acetaldehyde, followed by O-methylation and oxidation, considerations that led to the proposal of the 4-carboline arrangement of the three rings. The formation of *m*-nitroanisic acid mentioned above serves to locate the methoxyl group. Various syntheses of harmine, harmaline, and harman have demonstrated the correctness of these conclusions. The harmaline synthesis of Manske, Perkin, and Robinson [139] in 1927 removed the last point of uncertainty, the location of the alicyclic double bond in harmaline. A simpler synthesis of Späth and Lederer [140] has as a starting point the condensation of 3-methoxyphenylhydrazine with γ-amino-*n*-butyraldehydediethylacetal. Acetylation of the conden-

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[138] Perkin and Robinson, *J. Chem. Soc.*, **115**, 933, 967 (1919); Kermack, Perkin, and Robinson, *ibid.*, **119**, 1602 (1921).

[139] Manske, Perkin, and Robinson, *ibid.*, 1 (1927).

[140] Späth and Lederer, *Ber.*, **63**, 120, 2102 (1930); Akabori and Saito, *Ber.*, **63**, 2245 (1930).

sation product and closure of the pyridine ring with phosphorus pent-
oxide led to harmaline.
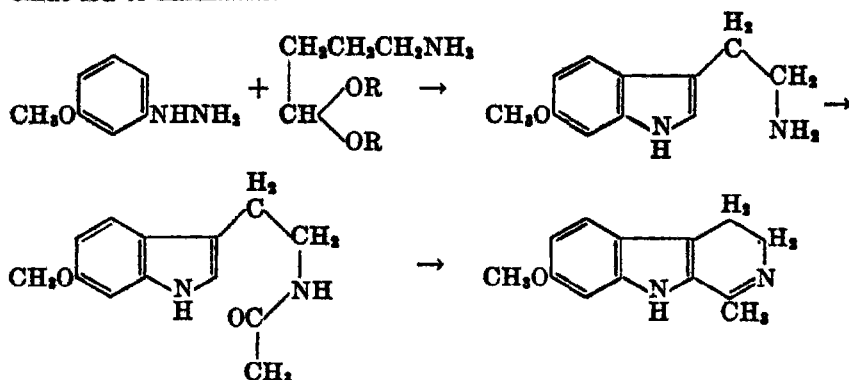
The phytochemical synthesis of the harman types suggested by Perkin
and Robinson has been supported experimentally by G. Hahn, through
the preparation of tetrahydroharman from cell-possible substances (tryp-
tamine and acetaldehyde) under physiological conditions (p. 1255).

While harmaline behaves toward alkylating agents like a base with
a tertiary pyridine nitrogen atom (formula III), the acetyl derivative
and the compounds resulting from the action of benzaldehyde or diazon-
ium salts are probably derived from the tautomeric form X.[129]
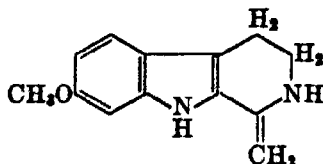
X. Harmaline, tautomeric form

Harmine and harmaline have a paralyzing action on the skeletal and
cardiac muscles; the use of *Peganum* seeds as a tapeworm remedy prob-
ably depends upon paralysis of the musculature of the worm. Harmine
has been found identical with banisterine, an alkaloid used in the treat-
ment of Parkinson's disease.

Physostigmine (Eserine). The fruit of the African vine *Physostigma
venenosum*, known as the Calabar or Esère bean, is used by the West
African natives for the administration of divine justice. An emetic sub-
stance in the seed hull often saves the accused person from fatal poison-
ing. The beans contain several alkaloids, of which physostigmine and
geneserine are the most important.

Early investigations of physostigmine, $C_{15}H_{21}O_2N_3$, established the
fact that two of the nitrogen atoms are tertiary and carry methyl groups.

The third nitrogen is split out as methylamine, together with carbon dioxide, by hydrolysis, and is present in a urethan grouping, a structural feature that has been found in no other alkaloid. The phenolic base resulting from the hydrolysis is known as eseroline (II), and can be converted back to physostigmine by the action of methyl isocyanate.[141] The ethyl ether of eseroline, known as eserethole (IV), has played an important part in structure determination. From zinc dust distillation of physostigmine, 1- and 2-methylindoles were obtained, but this violent degradation scarcely affords proof of the presence of the indole group.

Degradation of eseroline or eserethole methiodides (by heating in an atmosphere of carbon dioxide) results in physostigmol or its ethyl ether.[142] Physostigmol still contains the eseroline phenolic hydroxyl group and shows the color reactions characteristic of indoles. The relatively high yield obtained in the degradation indicates that the indole nucleus was

I. Physostigmine   II. Eseroline

III. Physostigmol   IV. Eserethole
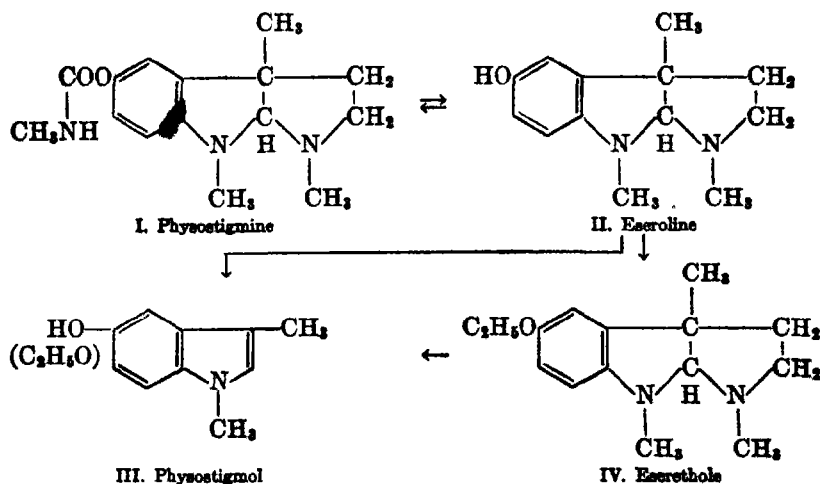
already present in eseroline, and therefore in physostigmine. The structure of physostigmol, and hence the position of the eseroline hydroxyl group, was established by Stedman's synthesis of physostigmol ethyl ether.[143] p-Ethoxyphenylmethylhydrazine (from reduction of nitrosomethyl-p-phenetidine) was condensed with α-ketoglutaric acid, giving the carboxymethylindoleacetic acid derivative V, from which, on decarboxylation, 5-ethoxy-1,3-dimethylindole, physostigmol ethyl ether (III), was obtained.

[141] Polonovski and Nitzberg, Bull. soc. chim., [4] 19, 27 (1916).

[143] Straus, Ann., 401, 350 (1913); 406, 332 (1916). Stedman, J. Chem. Soc., 125, 1373 (1924).

$$V$$

III
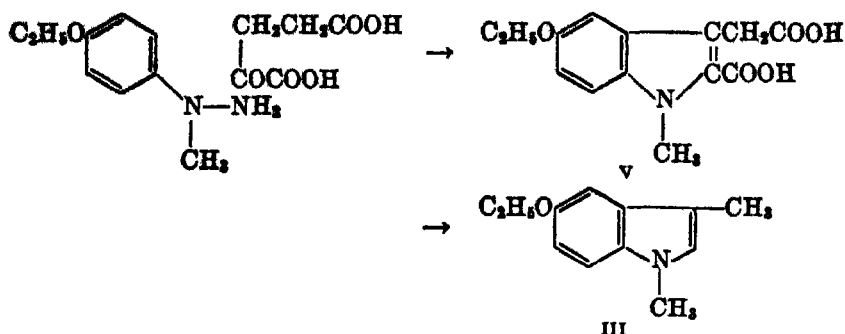
An alternative synthesis of physostigmol methyl ether by Späth involves condensation of p-methoxyphenylmethylhydrazine with propionaldehyde, followed by a Fischer indole ring closure.[143]

The physostigmine formula I was advanced by Stedman and Barger [144] on the basis of the known structure of physostigmol and the following considerations. Eserethole, on reduction, takes up two hydrogen atoms, which are used in opening a nitrogen-containing ring, for the product, dihydroeserethole (VI), is a secondary amine, in contrast to eserethole.

VI

VII
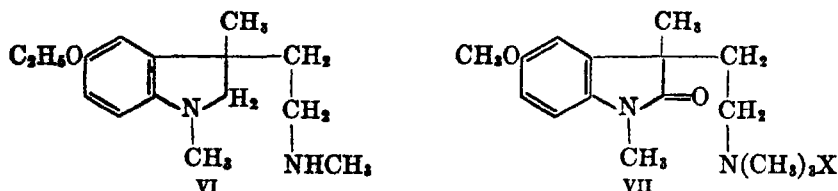
The further degradation of VI by exhaustive methylation supports the presence of the aminoethyl side chain. Existence of the angular methyl group was conclusively proved by King and Robinson's synthesis and resolution of the methine metho salts of formula VII, which were identical with those obtained from l-esermethole.

The physostigmine formula received final confirmation in the complete synthesis by Julian and Pikl.[145] This synthesis became feasible through the observation that the hydrogen atom on the 3-carbon of 1,3-dialkyloxindoles is so active that alkylation at this point takes place readily. The desired oxindole derivative was prepared by interaction of N-methyl-p-phenetidine and α-bromopropionyl bromide, followed by closure of the oxindole ring with aluminum chloride. The ring closure was accompanied by an undesired de-ethylation, therefore the 1,3-dimethyl-5-hydroxy-oxindole (VIII) was ethylated before further manipulation. The

[143] Späth and Brunner, Ber., 58, 518 (1925).
[144] Stedman and Barger, J. Chem. Soc., 127, 247 (1925).
[145] Julian and Pikl, J. Am. Chem. Soc., 57, 539, 563, 755 (1935).

ethoxy compound was condensed with chloroacetonitrile in the presence of sodium ethoxide, and the resulting nitrile (IX) was converted to the amine by catalytic hydrogenation. The primary amine was transformed to the secondary amine (XI) by Decker's method,* and the product was resolved into the d- and l-isomers at this point, since it was found that resolution at a later stage could not be accomplished. When l-1,3-dimethyl-5-ethoxy-3-β-methylaminoethyloxindole (XI) was reduced with sodium and alcohol, ring closure to l-eserethole occurred. The l-eserethole so obtained could be dealkylated (aluminum chloride) to l-eseroline, which was then converted by the Polonovski procedure,[141] with methyl isocyanate, to l-physostigmine.

N-Methyl-p-phenetidine

VIII. 1,3-Dimethyl-5-hydroxyoxindole

IX. 1,3-Dimethyl-5-ethoxyoxindolyl-3-acetonitrile

X. 1,3-Dimethyl-5-ethoxy-3-β-aminoethyloxindole

XI. 1,3-Dimethyl-5-ethoxy-3-β-methyl-aminoethyloxindole

XII. l-Eserethole

II. l-Eseroline

I. l-Physostigmine
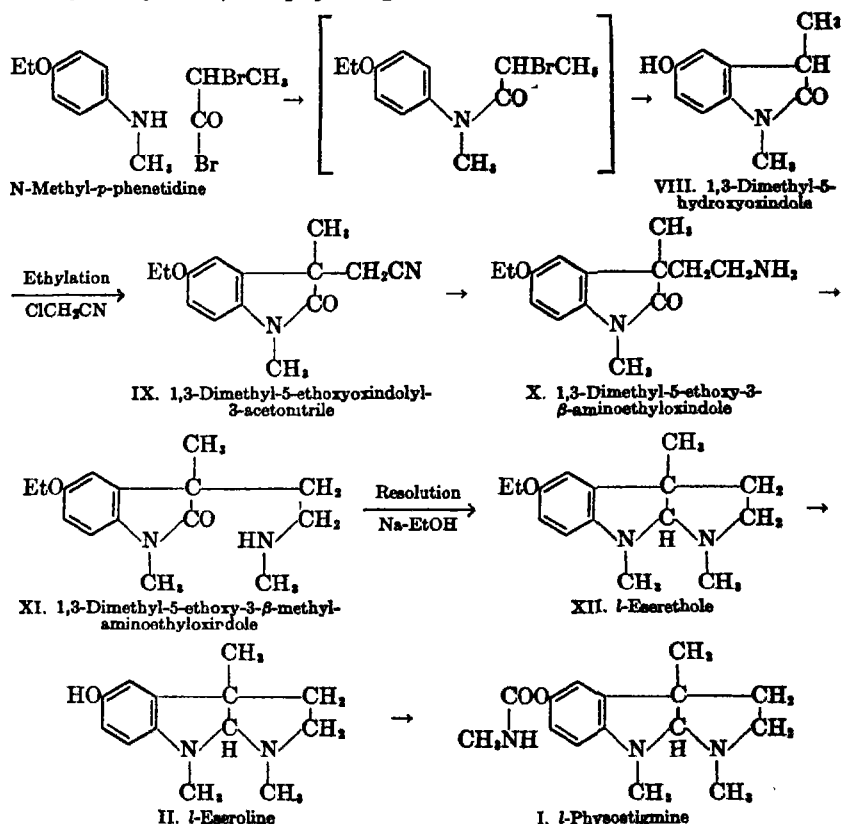
* Decker's method [Decker and Becker, *Ann.*, **395**, 362 (1913)] for the conversion of primary amines to secondary consists in condensation of the primary amine with an aldehyde (benzaldehyde), followed by addition of alkyl halide to the Schiff's base and subsequent hydrolysis:

$$RNH_2 + C_6H_5CHO \rightarrow RN{=}CHC_6H_5 \rightarrow R{-}N{\overset{CH_3}{\underset{I}{=}}}CHC_6H_5 \rightarrow RNHCH_3 + C_6H_5CHO + HI$$
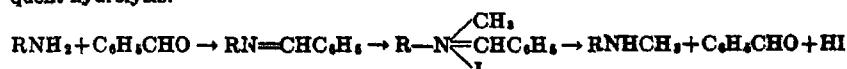
Geneserine, $C_{15}H_{21}O_3N_3$, contains one oxygen atom more than physostigmine. It can be reduced with ease to physostigmine, and conversely, is formed when physostigmine is treated with hydrogen peroxide, whence its nature as the N-oxide of physostigmine is evident. With the exception of an alkaloid of the lupine series,[146] it is the only natural alkaloid N-oxide that has been found.

Physostigmine is exceedingly poisonous, the fatal dose for man being in the neighborhood of 10 mg.; death usually results from respiratory paralysis. The alkaloid is used in opthalmic practice, and especially in the treatment of glaucoma. Geneserine is much less toxic, and is probably converted slowly to physostigmine in the body.

Yohimbine. The bark of the West African tree *Corynanthe yohimbe*, used by the natives as a powerful aphrodisiac, contains a number of related alkaloids, the most important of which is yohimbine. Quebrachine, from quebracho bark (Argentina), is identical with yohimbine.

Yohimbine, $C_{21}H_{26}O_3N_2$, is the methyl ester of yohimbic acid, $C_{19}H_{23}ON_2COOH$. The latter, on decarboxylation, is converted to yohimbol, which still contains the secondary alcoholic hydroxyl group known to be present in yohimbine. When yohimbine is heated with selenium, yobyrine $C_{19}H_{16}N_2$, tetrahydroyobyrine $C_{19}H_{20}N_2$, and ketodihydroyobyrine $C_{20}H_{16}ON_2$ are obtained. From the fragments ob-
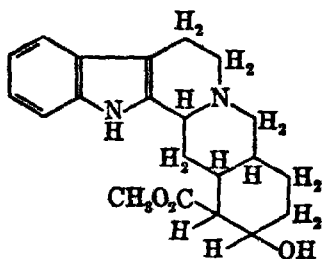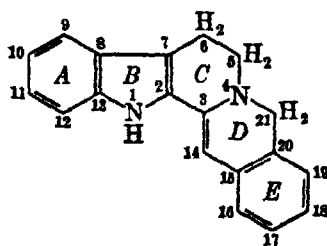
I. Yohimbine   II. Yobyrine

tained by degradation of these products, the yohimbine formula I has been derived.*

Yohimbic acid, with fused potassium hydroxide or zinc dust, yields harman (scission of ring D).[147] Ketodihydroyobyrine, on the other hand, when fused with alkali, suffers breakage of ring D at a different point, and gives norharman and 2,3-dimethylbenzoic acid.[148]

[146] Couch, *J. Am. Chem. Soc.*, **58**, 1296 (1936).

* The position of the alcoholic hydroxyl group is not known with certainty.

[147] Warnat, *Ber.*, **60**, 1118 (1927); Barger and Scholz, *J. Chem. Soc.*, 614 (1933).

[148] Mendlik and Wibaut, *Rec. trav. chim.*, **50**, 91 (1931); Barger and Scholz, *Helv. Chim Acta*, **16**, 1343 (1933).
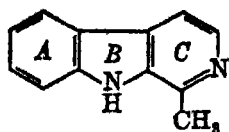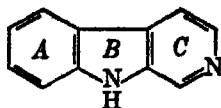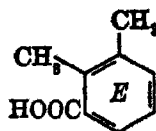
Harman   Norharman   2,3-Dimethylbenzoic acid

When tetrahydroyobyrine is oxidized with nitric acid, ring D survives intact and appears as berberonic acid. Ring D is likewise found, together with ring E, in the form of isoquinoline, from zinc dust degradation of yohimbic acid.[149]

The position of the carboxyl group on ring E may be assumed from the formation of dimethylbenzoic acid mentioned above, and from the appearance of m-toluic acid when yohimbine or yohimbic acid is treated with superheated steam.
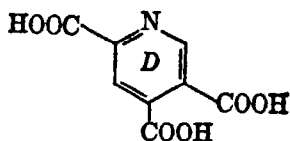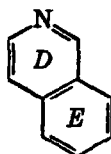
Berberonic acid   Isoquinoline m-Toluic acid
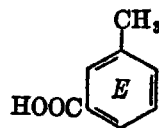
Ring E can also be obtained as phthalic acid by oxidation of yobyrine. The indole grouping, rings A and B, was obtained by Barger and Scholz as 3-ethylindole from potash fusion of yohimbic acid, and ring A with ring B opened appears in oxalylanthranilic acid, which results from permanganate oxidation of yohimbine.[150]
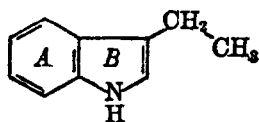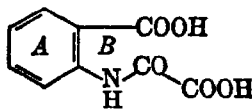
3-Ethylindole   Oxalylanthranilic acid

The position of the yohimbine alcoholic hydroxyl group is still somewhat uncertain. Scholz favors the 14-position, as accounting best for the difficulty observed in the hydrogenation of apoyohimbine, a dehydration product resulting from the action of sulfuric acid on yohimbine. Hahn regards positions -17, -18, or -19 as possible, excluding -14 on the basis of the quantitative alkali degradation of tetradehydroyohimbine into m-toluic acid and harman, whereby carbon atom-14 appears as the

[149] Winterstein and Walter, *Helv. Chim. Acta*, **10**, 577 (1927).
[150] Späth and Bretschneider, *Ber.*, **63**, 2097 (1930).

harman methyl group.[151]   In formula I the hydroxyl group has been placed on carbon-17 because the remarkable synthesis of the yohimbine skeleton by Hahn and Werner, accomplished almost entirely under physiological conditions, makes it seem possible that the plant synthesizes the alkaloid by a parallel process, a synthesis that could scarcely succeed were the hydroxyl group at a position other than that shown.[152]

Yohimbine is used as an aphrodisiac. It promotes sexual desire in both male and female through dilation of the blood vessels of the genital organs, and also stimulates the sexual centers of the spinal cord.

Strychnos Alkaloids.[153, 154]   The alkaloids of *Strychnos nux-vomica* and of Ignatius beans (*S. Ignatii*), strychnine, brucine, and vomicine,
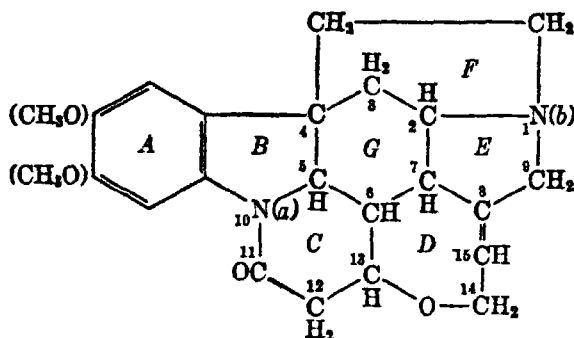
I. Strychnine (Brucine)

present a structural problem of such complexity that only the salient features can be mentioned.   In spite of the intensive researches being carried on at present in the laboratories of Leuchs, Robinson, Wieland, and others, it is not yet possible to present for strychnine a structural formula that is certain in every detail.   The most recent proposal (I) may serve for discussion; Leuchs favors the linkage of the C_2H_4 group to carbon-3.

Brucine, $C_{23}H_{26}O_4N_2$, is a dimethoxy derivative of strychnine, $C_{21}H_{22}O_2N_2$, and behaves like it in most reactions not involving the aromatic nucleus. The position of the brucine methoxyl groups, already deduced from color reactions, is confirmed by Späth and Bretschneider's oxidation of strychnine to N-oxalylanthranilic acid, of brucine to 4,5-dimethoxy-N-oxalylanthranilic acid (II).

[151] Hahn, Kappes, and Ludewig, *Ber.*, **67**, 686 (1934).
[152] Hahn and Werner, *Ann.*, **520**, 123 (1935).
[153] R. Robinson, "Bakerian Lecture," *Proc. Roy. Soc. (London)*, **A130**, 431 (1931). *Annual Review of Biochemistry* (1933), Vol. II, p. 444; (1935), Vol. IV, p. 497. Späth, *ibid.* (1937), Vol. VI, p. 528. Small, *ibid.* (1939), Vol. VIII, p. 478.
[154] Seka, "Alkaloide," Urban and Schwarzenberg, Berlin (1933).
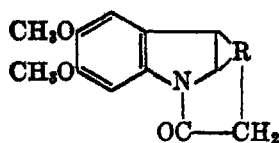
Ia. Brucine
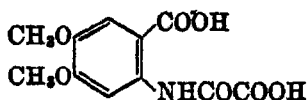
II. 4,5-Dimethoxy-N-oxalylanthranilic acid

One of the two nitrogen atoms of strychnine, N(a), has no basic properties, and is in a cyclic amido group. By hydrolysis at this point, strychnine is converted into strychnic acid (III). By electrolytic reduction at the amido carbonyl group in strychnine, strychnidine (IV) and tetrahydrostrychnine (V) are formed.
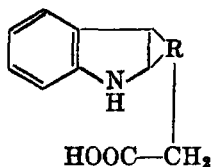
III. Strychnic acid
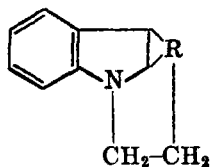
IV. Strychnidine
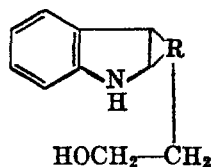
V. Tetrahydrostrychnine

These products still contain a double linkage, whose presence, as in strychnine itself, may be demonstrated by catalytic hydrogenation. The strychnine amido group forms part of the system R—N(a)—COCH₂— in which R is a benzene ring with a free *para*-position; this is deduced from the fact that strychnine, but not strychnidine, condenses with benzaldehyde to give a colored benzylidene derivative. Furthermore, strychnine and strychnidine behave as though related as acylaniline to alkylaniline: strychnine does not give coupling reactions with diazonium salts, but strychnidine yields with diazobenzenesulfonic acid an azo compound that is an indicator resembling methyl orange. The aminostrychnidine resulting from reduction of the azo compound resembles *p*-aminodimethylaniline, and gives analogs of the toluylene dyes.

The second, basic nitrogen atom, N(b), carries no methyl group; it is tertiary, even in hydrogenated derivatives, and therefore is not in an —N=C— group. When strychnine methiodide is treated with alkali or silver oxide, strychnic acid methohydroxide is formed, and this loses a molecule of water to yield the betaine, methylstrychnine, a secondary base [N(a)].

Strychnine methiodide

Strychnic acid methohydroxide

Methylstrychnine

Dimethylstrychnine
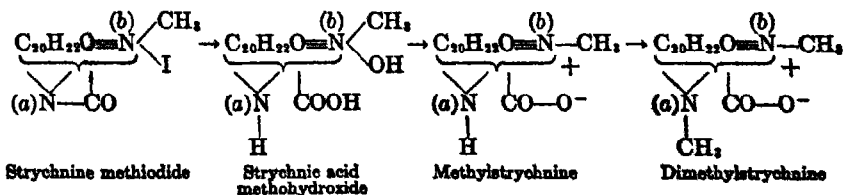
Methylstrychnine reacts with hydriodic acid to give strychnic acid methiodide; with methyl iodide it yields dimethylstrychnine (N-methyl-strychnic acid methylbetaine), in which $N(a)$ is tertiary and basic.

One of the strychnine oxygen atoms is accounted for in the amido carbonyl group; the second oxygen is indifferent to all reagents for the hydroxyl or carbonyl group, and must be present in an ether linkage. By very vigorous reduction, phosphorus and hydriodic acid, the ether oxygen may be eliminated (desoxystrychnine) without loss of any other portion of the molecule, a fact that indicates a cyclic ether structure. Strychnidine does not undergo this type of change. Strychnine and dihydrostrychnine, but not the strychnidines, suffer isomerization under the influence of basic catalysts, giving isostrychnine and dihydroiso-strychnine. The members of the *iso* series contain an alcoholic hydroxyl group, which can be formed only by a change at the ether oxygen; a shrinkage of ring *D* has been suggested to explain the rearrangement. The extreme resistance of the ether oxygen in strychnidine compared
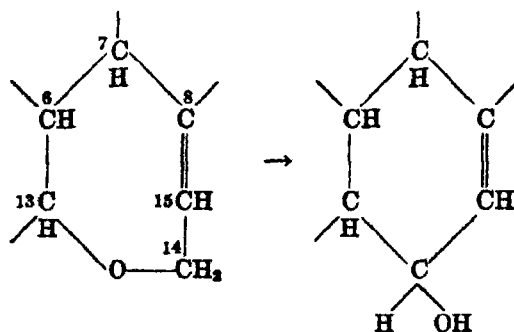
with that in strychnine in the reactions mentioned makes it probable that this oxygen is not far removed from —$N(a)$—CO—, as in the system —$N(a)$—CO—CH_2—CH—O—. This arrangement is confirmed by the results from the Beckmann rearrangement of isonitrosostrychnine.[155] Treatment of isonitrosostrychnine hydrochloride with thionyl chloride yields a compound (VII), isomeric with the isonitroso derivative. This rearrangement product hydrolyzes with great ease, losing carbon dioxide and hydrogen cyanide, to give the aldehyde-alcohol VIII, in which one oxygen atom obviously is that of the former cyclic ether structure.[*]

Evidence concerning the propinquity of $N(b)$, the double linkage, and the ether oxygen has been found in the Leuchs degradation. When

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[155] Wieland and Kaziro, *Ann.*, **506**, 60 (1933).

[*] In the original publication the ether-linked oxygen is shown as part of a six-membered ring. This view has been abandoned by Wieland (private communication).

VI   VII   VIII
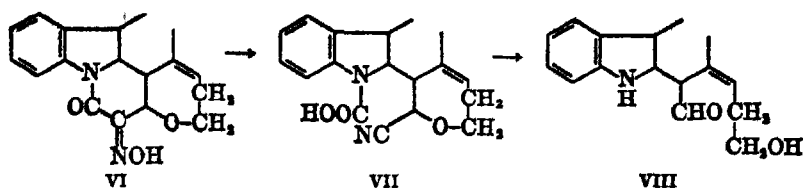
strychnine is oxidized with permanganate in acetone, strychninonic and dihydrostrychninonic acids are obtained. Strychninonic acid can be reduced to strychninolic acid, which decomposes in the presence of sodium hydroxide into strychninolone and glycolic acid. In the brucine series the corresponding products obtained are brucinonic and brucinolic acids, and brucinolone. These changes are represented by part-formulas IX–XI.

I Strychnine   IX. Strychninonic acid

X. Strychninolic acid   XI. Strychninolone   Glycolic acid
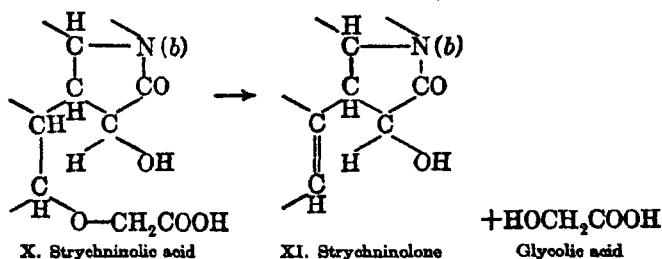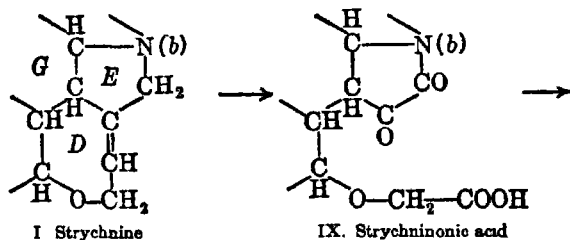
Support for this mechanism is seen in the fact that strychninolic acid contains no double linkage, but strychninolone does. The observation that brucinolic acids in which N(a)CO has been reduced to N(a)CH$_2$— do not undergo the glycolic acid decomposition constitutes an additional argument for the relative positions of N(a)CO and the ether oxygen. The presence of the N(b)CO group in strychninonic acid (and hence of —N(b)CH$_2$— in strychnine) is deduced from the fact that strychninonic esters are not basic, and from the appearance of dihydrostrychninonic acid mentioned above. This acid (XIII) seems to be a diastereoisomer of strychninolic acid, X, and can be oxidized to strychninonic acid, IX.

A mechanism proposed for its formation from strychnine postulates the changes I → XII → XIII:
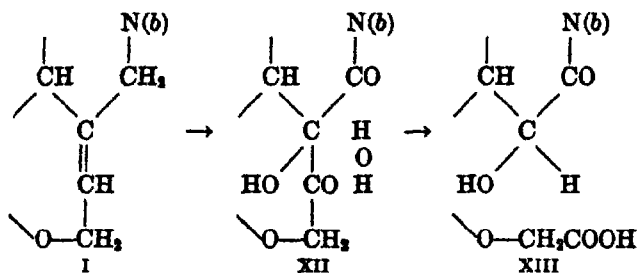
the last of which, XII to XIII, is plausible only if XII contains the group —N(b)CO—.

Oxidation of brucinonic acid with hydrogen peroxide yields a product from which the 9-carbon atom has been lost as carbon dioxide, and which has properties indicating that N(b) must have been in a five-membered ring, E (Leuchs). Evidence for the existence of ring G has been difficult to obtain, but its presence is made probable by the appearance of carbazole, containing rings A, B, and G, in vigorous degradations (zinc dust distillation) of strychnine. In this reaction rings A and B are also revealed as indole.

Carbazole
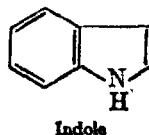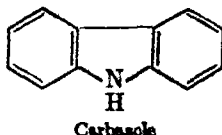
Indole

The indole nucleus has been identified more acceptably through nitric acid oxidation of strychnine. This process leads to dinitrostrychol-carboxylic acid (XIV), which can be further degraded by the Curtius method through the azide and urethan to dinitroisatin (Robinson).
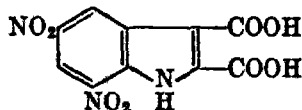
XIV. Dinitrostrycholcarboxylic acid
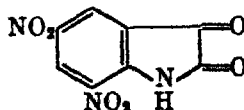
XV. Dinitroisatin

By destruction of four carbon atoms of the aromatic nucleus in strychnine and brucine, derivatives of the hypothetical base nucine are obtained, and similarly from strychnidine and brucidine, derivatives of nucidine. Destruction of the entire aromatic nucleus yields the aponucines or aponucidines.

XVI. Nucine   XVII. Nucidine   XVIII. Aponucine
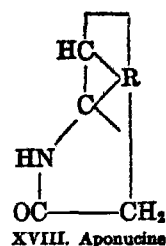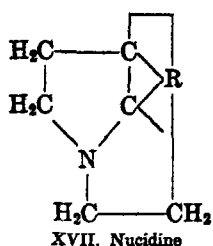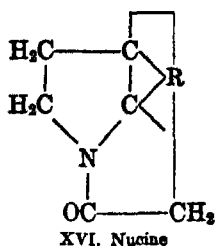
Oxidation of strychnine with chromic acid gives the "Wieland-Münster C_{17} acid," 2,3-diketonucine dihydrate. From brucine, "Hanssen's C_{19} acid" is first obtained, and can be converted to the Wieland-Münster acid. This last loses a carbon atom with alkaline hydrogen peroxide, and closes the lactam ring, yielding "Hanssen's C_{16} acid," aponucine-carboxylic acid.

I. Strychnine
$C_{21}H_{22}O_2N_2$

Ia. Brucine
$C_{23}H_{26}O_4N_2$

XIX. Wieland-Münster acid
$C_{17}H_{22}O_6N_2$

XX. Hanssen's C_{19} acid
$C_{19}H_{22}O_6N_2$

XXI. Hanssen's C_{16} acid
$C_{16}H_{20}O_4N_2$

XXII. Aponucine
$C_{15}H_{20}O_2N_2$
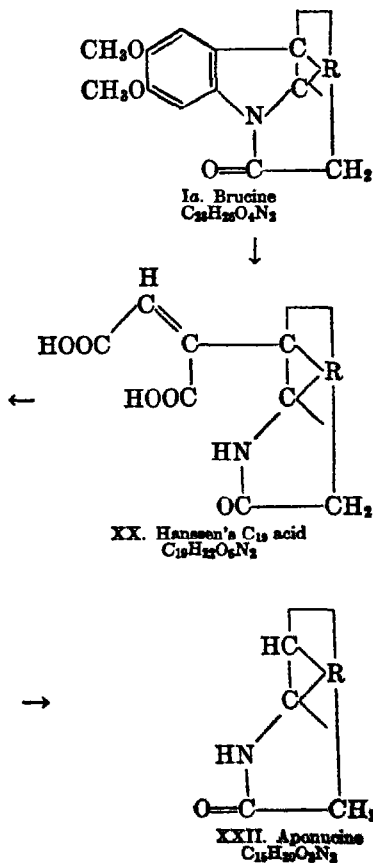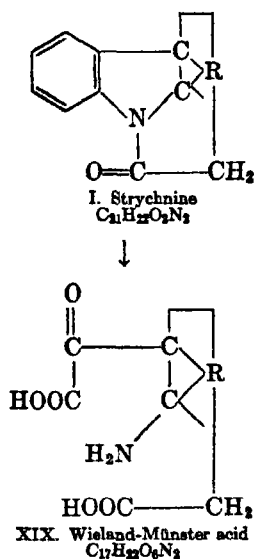
Information concerning the C_2H_4 chain attached to N(b) has been most difficult to obtain. An erroneous interpretation of the bromination of diketonucidine (XXIII or XXIV) excluded for many years linkage to C-4, and during this period C-3 was favored by Leuchs, C-5 by Robinson.[154]

XXIII. Diketonucidine   XXIV. Diketonucidine
(Old formula)   (New formula)
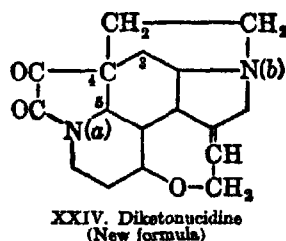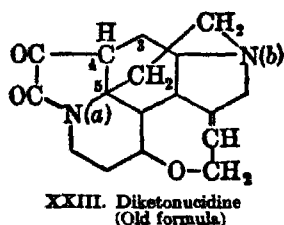
Diketonucidine, an oxidation product of strychnidine (IV), reacts with one mole of bromine (water) to give monobromodiketonucidine hydrate. It was believed that substitution of the active hydrogen α to the carbonyl group (XXIII) had taken place, and since C-4 carried a hydrogen atom, the chain could not be attached to it. Independent studies of the bromination by these two investigators [155] have recently shown that the bromination probably involves addition of HOBr at the double bond (C-8, C-15); dihydrodiketonucidine does not react with bromine. This new evidence makes C-4 available as an attachment point for the two-carbon chain (as originally suggested by Menon and Robinson in 1932).[157] There is further strong evidence that this chain is at the β-position of the indole nucleus (4-position of strychnine) in the discovery that degradation of strychnine and some of its derivatives with strong alkali gives tryptamine.[158]

Tryptamine
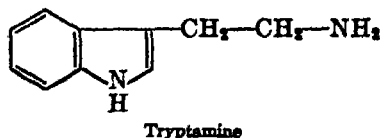
Vomicine, $C_{22}H_{24}O_4N_2$, resembles strychnine and brucine in some of its reactions, but on hydrolysis yields the amino acid vomicinic acid (XXVI), which contains a new phenolic hydroxyl group. This phenolic hydroxyl is also generated when vomicine is reduced to vomicidine by the electrolytic method. From reduction studies, and oxidative and pyro-

[154] Holmes and Robinson, J. Chem. Soc., 603 (1939); Leuchs and Grunow, Ber., 72, 679 (1939); Leuchs, Ber., 72, 1588 (1939).
[157] Menon and Robinson, J. Chem. Soc., 780 (1932).
[158] Kotake, Proc. Imp. Acad. Tokyo, 12, 99 (1936); Clemo, J. Chem. Soc., 1695 (1936).

lytic degradations, Wieland [159] has developed the incomplete formula XXV for vomicine.
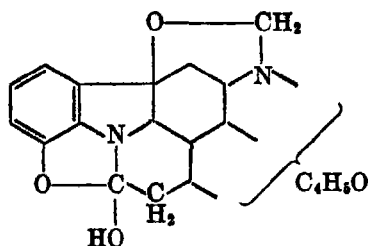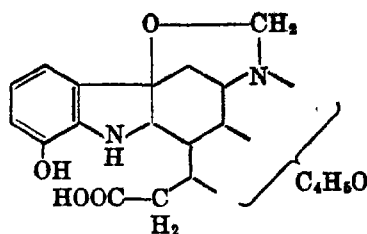
XXV. Vomicine   XXVI. Vomicinic acid

Strychnine, because of its extremely high toxicity, is widely used as a pest exterminator. In therapeutic doses it acts to stimulate the respiratory and vasomotor nerve centers; in larger amounts it acts on the spinal cord to cause high reflex irritability, involving all the muscles of the body. Convulsions or tetanus may result from the slightest stimulus, death usually following from respiratory exhaustion. Brucine has about one-eighth the toxicity of strychnine.

Ergot Alkaloids. [160] The mycelia of the fungus *Claviceps purpurea*, from diseased rye and other *Gramineae*, constitute the important drug known as ergot. Ergot has yielded, in addition to such simple putrefaction bases as histamine, tyramine, cadaverine, and putrescine, a series of exceedingly complex alkaloids, whose structures have now been largely elucidated. The ten most important ergot bases are closely related, and may for the most part be discussed as a group. The longest known pair, ergotoxine * and ergotinine, $C_{35}H_{39}O_5N_5$, are isomeric and interconvertible. Ergotinine is transformed to ergotoxine by boiling alcoholic phosphoric acid, and the reverse change takes place in boiling methanol or with acetic anhydride. Ergotamine, $C_{33}H_{35}O_5N_5$, and ergotaminine are similarly related. Ergonovine, $C_{19}H_{23}O_2N_3$, was discovered during the period 1932–1934 by four independent investigators, who proposed the names ergobasine, ergometrine, ergostetrine, and ergotocin. It is at present believed to be the most active constituent of ergot in oxytocic effect. Ergonovine is possibly accompanied by its isomer, ergometrinine, which is a transformation product. The most recently discovered pairs are ergosine and ergosinine, [161] $C_{30}H_{37}O_5N_5$, and ergocristine and ergo-

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[159] Wieland and Horner, *Ann.*, **528**, 73 (1938).

[160] Barger, "Ergot and Ergotism," Gurney and Jackson, London (1931).

* Ergotoxine was long considered to be $C_{35}H_{41}O_6N_5$, but the pair are now believed to be isomerides; the discrepancy may be due to analytical difficulty, or to a firmly bound molecule of hydrate water.

[161] Smith and Timmis, *J. Chem. Soc.*, 396 (1937).

cristinine,[162] $C_{35}H_{39}O_5N_5$. The fact that in each of these pairs one member is physiologically active and levorotatory (ergotoxine, ergotamine, ergonovine, etc.), while the other member is relatively inert and strongly dextrorotatory, suggests that a similar change of structure is involved in the reversible transformation of all the isomers.

The members of the pairs show a tendency to crystallize together; the equimolecular mixture of ergosine and ergosinine was for some time thought to be a new alkaloid ("ergoclavine"), as was that of ergotamine and ergotaminine ("sensibamine"). The nature of pseudoergotinine is still uncertain; it can be transformed to ergotinine, or to ergotoxine, and is possibly a mixture.

Early work on the series consisted largely of degradations so violent as to yield little information. From destructive distillation of ergotoxine or ergotinine, isobutyrylformamide $(CH_3)_2CHCOCONH_2$ was isolated. Oxidation with permanganate or nitric acid gave benzoic and p-nitrobenzoic acids respectively, and a tribasic acid, $C_{14}H_9O_8N$.

The key to the structure of the ergot group lies in hydrolytic procedures which are known to split amide or peptide linkages, viz.: acid hydrolysis, alkaline hydrolysis, and sodium-butyl alcohol reduction.[163, 164] Hydrolysis in aqueous alkali led to the isolation of lysergic acid, $C_{16}H_{16}O_2N_2$.[164] Smith and Timmis then showed that ergine, a product previously isolated by them, was the amide of lysergic acid.[165] All the known alkaloids of ergot have yielded either lysergic acid or ergine. The hydrolysis of the ergocristine pair has not been carried out. Lysergic acid is the only group common to all the members, and the transformations, and changes in physiological action, must be due to changes in the lysergic acid portion of the molecule.

Extensive work on the hydrolysis of the ergot alkaloids indicates that they are probably constituted as follows:

Ergotoxine and ergotinine consist of d-lysergic and d-isolysergic acids respectively, in combination with d-proline, l-phenylalanine, and α-hydroxyvaline. The α-hydroxyvaline is not isolated as such, but in the form of its decomposition product, isobutyrylformic acid (dimethylpyruvic acid). The presence of the *dextro* form of proline, which occurs elsewhere in nature only in the *levo* form, is noteworthy.

Ergotamine and ergotaminine contain α-hydroxyalanine (isolated as pyruvic acid) in place of the α-hydroxyvaline of the preceding pair.

[162] Stoll and Burckhardt, *Z. physiol. Chem.*, **250**, 1 (1937); **251**, 287 (1938).
[163] Jacobs and Craig, *J. Biol. Chem.*, **106**, 393 (1934); *J. Am. Chem. Soc.*, **57**, 383, 960 (1935); Smith and Timmis, *J. Chem. Soc.*, 763 (1932).
[164] Jacobs and Craig, *J. Biol. Chem.*, **104**, 547 (1934).
[165] Smith and Timmis, *J. Chem. Soc.*, 674 (1934).

Ergosine and ergosinine have, in addition to the lysergic acid portion, α-hydroxyalanine, *d*-proline, and *l*-leucine.

Ergonovine and ergometrinine, the simplest of the group, are the hydroxyisopropylamides of lysergic and isolysergic acids, and give *d*-2-aminopropanol on hydrolysis.

$$CH_2—CH_2$$
$$|\qquad|$$
$$CH_2\quad CHCOOH$$
$$\diagdown N\diagup$$
$$\quad H$$
Proline

$$H_2$$
$$C$$
$$\bigcirc CHCOOH$$
$$|$$
$$NH_2$$
Phenylalanine

$$(CH_3)_2CHCH_2CH\diagup{}^{NH_2}_{\diagdown COOH}$$
Leucine

$$CH_3\diagdown\quad{}^{NH_2}_{|}$$
$$\qquad CHCCOOH$$
$$CH_3\diagup\quad|$$
$$\qquad\quad OH$$
α-Hydroxyvaline

$$\quad NH_2$$
$$\quad |$$
$$CH_3CCOOH$$
$$\quad |$$
$$\quad OH$$
α-Hydroxyalanine
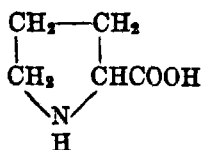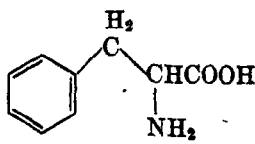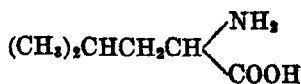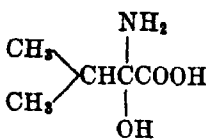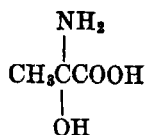
$$CH_3\diagdown$$
$$\qquad CHCH_2OH$$
$$NH_2\diagup$$
2-Aminopropanol-1

These amino acids, in the combinations mentioned above, are believed to be joined in amide linkages with each other and with the carboxylic acid group of lysergic or isolysergic acid in the several alkaloids. A proposal,[166] for example, for the structure of ergotoxine is shown in I. Ergotinine differs only in containing an isolysergic acid group (see below).

$$CH_3\quad CH_3$$
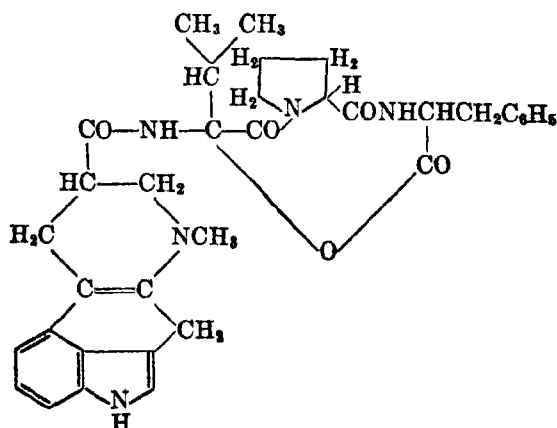
I. Ergotoxine

With the identification of the amino acid constituents, it is apparent that the constitutional question is essentially that of lysergic acid. This

[166] Craig, Shedlovaky, Gould, and Jacobs, *J. Biol. Chem.*, **125**, 289 (1938).

acid is optically active, contains an N-methyl group but no methoxyl groups, and is monobasic. It also forms stable salts with one equivalent of acid. It contains an easily reducible double bond, which must be near the carboxyl group, for dihydrolysergic acid is a weaker acid, and loses carbon dioxide with more difficulty than lysergic acid. The double bond must be involved in the isomerism of the alkaloids, for dihydrolysergic acid, like the dihydro alkaloids, cannot be isomerized. Absorption spectra indicate that the unsaturated center is conjugated with the indole nucleus which is revealed in the form of a dimethylindole from alkali fusion of dihydrolysergic acid.[167]

In earlier speculations, these considerations, as well as the probable biogenetic relationship of lysergic acid to tryptophan, led to the proposal of a 4-carboline (see p. 1228) type formula for lysergic acid. Synthetic analogs of the proposed formula, however, gave no color test [168] with dimethylaminobenzaldehyde, and the 4-carboline formula is also incapable of explaining the appearance of 1-methyl-5-aminonaphthalene (II) in alkali fusion of dihydrolysergic acid.

The tribasic acid $C_{14}H_9O_9N$, obtained from nitric acid oxidation of ergotinine or lysergic acid, yields quinoline on soda-lime distillation, and appears to be an N-methylquinoliniumbetainetricarboxylic acid, for which III is a possible formula.

II   III
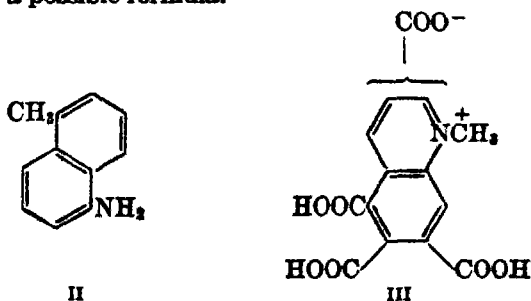
These facts led Jacobs and Craig to advance two formulas for lysergic acid, in which the chief uncertainty was the positions of the carboxylic acid group and of the double bond.[169] Later evidence on the location of these features resulted in formulas IV and V for lysergic acid and isolysergic acid respectively.[168]

Cleavage of ring B at 1,2 and 2,3 and of ring D at 5,6 and 8,9 accounts for the 1-methyl-5-aminonaphthalene, and oxidative cleavage of rings A and B explains the formation of the acid III.

[167] Jacobs and Craig, *ibid.*, **128**, 715 (1939).
[168] Jacobs and Craig, *Science*, **82**, 421 (1935).
[169] Jacobs and Craig, *J. Biol. Chem.*, **115**, 227 (1936).

COOH
HC—5—CH₂
H₂C 9 10 D 6 NCH₃
C=C 5
11 C 5 4CH₂
16
12 A 15 B 3
13 14 1 2
N
H

IV. Lysergic acid

COOH
HO—CH₂
HC NCH₃
C—CH
CH₂
N
H
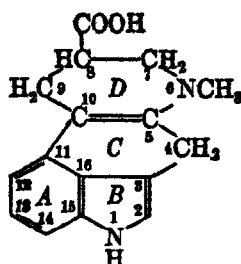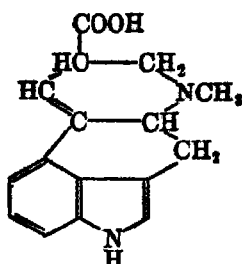
V. Isolysergic acid

The Double Bond. The basic skeleton for lysergic acid is supported by the synthesis of 6,8-dimethylergoline (racemic) VI, which is, except for optical properties, practically identical with a base prepared from dihydrolysergic acid (by reduction of VII).[170]

CH₃
H H₂
H₂ NCH₃
H H
H₃
N
H
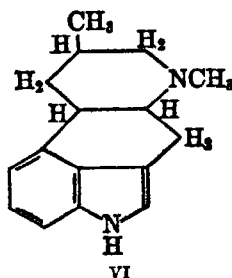
VI

Accepting this skeleton, the only possible positions for the double bond are (4,5), (5,10), and (10,9), for (9,8) and (8,7) are not in conjugation with the indole nucleus. The (4,5)(5,10) positions for the isomeric acids would place the unsaturation equidistant from the basic group in the isomers, which is not in accord with evidence derived from dissociation constants. These indicate that in ergometrinine the double bond must be further from nitrogen than in ergonovine, a condition met only by the 10,9-position for isolysergic, and the 5,10-position for lysergic acid (formulas IV and V).

The Carboxylic Acid Group. To account for the quinolinebetaine-carboxylic acid, and for the optical activity, the carboxylic group can occupy only positions 4, 9, 8, or 7. Of these, 9 seems eliminated, since shift of the double bond from 5,10 to 10,9 should cause racemization. Position 4 would make dihydrolysergic acid a substituted indoleacetic acid, which does not accord with its relative stability towards loss of carbon dioxide on pyrolysis. Of 7 and 8, the latter is favored by a study of dissociation constants. Further evidence is found in the pyrolysis of dihydrolysergic acid, which yields a non-basic, unsaturated compound,

[170] Jacobs and Gould, *ibid.*, **130**, 399 (1939).

believed to be a cyclic amide as shown in part-formula VII. It is known that β-amino acids, in contrast to α-amino acids, decompose into unsaturated acids and ammonia. The analogous behavior of dihydrolysergic acid points to the β-amino structure.
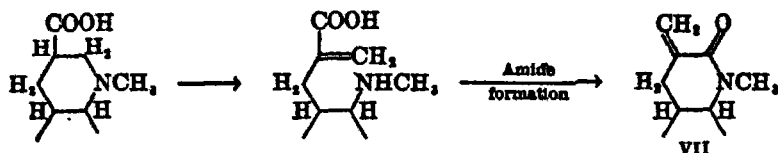
The partial synthesis of ergonovine and its isomer ergometrinine [171] confirms the concepts concerning the isomerism of the ergot alkaloids. Hydrolysis of the ergot alkaloids with hydrazine hydrate proceeds with isomerization and racemization, to give racemic isolysergic acid hydrazide. Condensation of this with d-2-aminopropanol-1 gave a mixture of d-isolysergic-d-isopropanolamide and l-isolysergic-d-isopropanolamide. The former is ergometrinine. By isomerization of the mixed isolysergic-isopropanolamides with acid, the corresponding mixed d,d- and l,d-lysergicisopropanolamides were obtained, of which d-lysergic-d-isopropanolamide was identical with ergonovine.

Ergotoxine, ergotamine, and ergonovine are characterized by their vasoconstrictor action and their power to cause a gangrenous condition of the type observed in the gangrene epidemics (ergotism, St. Anthony's fire) known since the Middle Ages to result from the consumption of bread made with diseased rye.

IMIDAZOLE AND QUINAZOLINE ALKALOIDS: PILOCARPINE AND VASICINE

Jaborandi Alkaloids. The leaves of *Pilocarpus jaborandi* contain several related alkaloids, of which pilocarpine, $C_{11}H_{16}O_2N_2$, is the most important. This alkaloid was shown in the early work of Jowett and of Pinner [172] to be a mono-acid tertiary base containing a lactone group and an imidazole nucleus, but only in recent years has its structure been conclusively demonstrated.
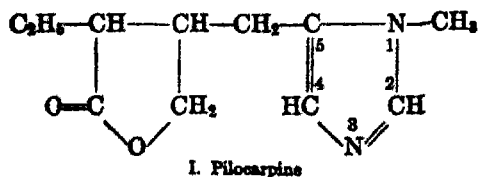
I. Pilocarpine

[171] Stoll and Hofmann, *Z. physiol. Chem.*, **251**, 155 (1938); **250**, 7 (1937).

[172] Jowett, *J. Chem. Soc.*, **83**, 438 (1903); Pinner and Schwarz, *Ber.*, **35**, 192, 2241 (1902)

Pilocarpine undergoes isomerization with ease to yield isopilocarpine, a base that also occurs in jaborandi, and that has served for much of the structural investigation. The arrangement of the two nitrogen atoms is indicated by the appearance of methylurea in oxidations. By distillation of either base with soda-lime, 1-methylimidazole, 1,5-dimethylimidazole, and 1-methyl-5-amylimidazole are obtained.[171]

| NHCH₃ | CH₃(CH₂)₄C——NCH₃ | CH₃C——NCH₃ |

Methylurea   1-Methyl-5-n-amylimidazole   1,5-Dimethylimidazole
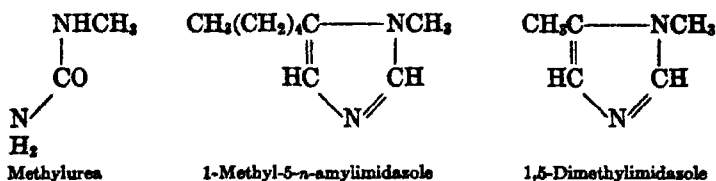
As fragments from the nitrogen-free portion of isopilocarpine, Jowett, by permanganate oxidation, obtained isopilopic and homoisopilopic acids; * the same acids likewise appear in oxidations of pilocarpine as a result of rearrangement.

II. Isopilopic acid   III. Homoisopilopic acid

IV. Ethyltricarballylic acid
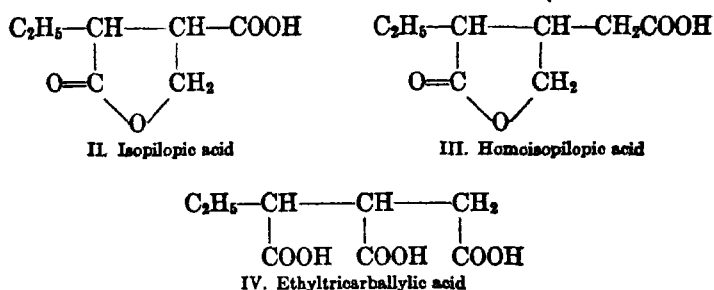
The constitution of homoisopilopic acid is deduced from the transformation to ethyltricarballylic acid by alkali fusion. Chichibabin and Preobrashenski [174] demonstrated the structure of isopilopic acid by synthesis, and prepared the diastereoisomeric pilopic acid corresponding to the nitrogen-free portion of pilocarpine. Pilopic acid undergoes rearrangement with extreme ease to isopilopic acid, so that the latter is always obtained from pilocarpine oxidation. By ozonolysis of pilocarpine and isopilocarpine, isomeric homopilopic acid amides are obtained, showing that the two alkaloids differ only in the stereochemical arrangement of this part of the molecule.[175]

The synthesis of pilocarpine and isopilocarpine was accomplished in

[171] Akabori and Numano, Ber., 66, 159 (1933).
* Originally designated as pilopic and homopilopic acids.
[174] Chichibabin and Preobrashenski, Ber., 63, 460 (1930).
[175] Langenbeck, Ber., 57, 2072 (1924).

1933 by Preobrashenski.[176] d-Homoisopilopyl chloride was converted by the diazomethane reaction to chloromethyl homoisopilopyl ketone (V). By Gabriel's reaction, V yielded the corresponding aminomethyl ketone, on which the imidazole nucleus was built by heating with potassium thiocyanate. Treatment of the resulting mercapto-isopilocarpidine with ferric chloride gave isopilocarpidine (an isomerization product of the jaborandi alkaloid pilocarpidine), which on methylation yielded isopilocarpine. Pilocarpine was prepared similarly starting from d-homopilopic acid.

C_2H_5—CH——CH—CH$_2$CO

CH_2 → Amine →

O=C   CH$_2$ Cl

V. Chloromethyl homoisopilopyl ketone

C_2H_5—CH——CH—CH$_2$—C——NH

O=C   CH$_2$ HC   CSH
              N

VI. Mercapto-isopilocarpidine

C_2H_5—CH——CH—CH$_2$—C——NH

O=C   CH$_2$ HC   CH
              N

VII. Isopilocarpidine (Pilocarpidine)

→

C_2H_5—CH——CH—CH$_2$—C——N—CH$_3$

O=C   CH$_2$ HC   CH
              N

I. Isopilocarpine (Pilocarpine)
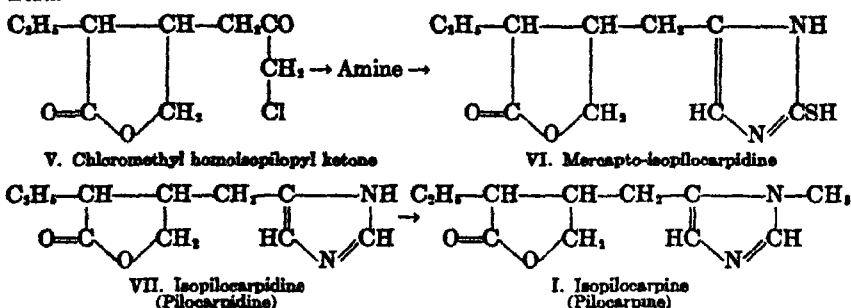
Pilocarpine acts on the nerve endings of the secretory cells, causing increased secretion of sweat, saliva, and tears. It is used as a diaphoretic, and in optical surgery to cause myosis and to reduce intraocular pressure.

Vasicine. Vasicine, also known as peganine, was first found in the Himalayan plant *Adhatoda vasica*, and was later isolated from the mother liquors from preparation of the harmala alkaloids. *Adhatoda* is used in India as a fish poison, insecticide, and for the relief of asthma. Although vasicine contains an asymmetric carbon atom, it is optically inactive. This phenomenon is encountered rarely in the alkaloid series, and in vasicine the inactive base is formed by racemization during the isolation of the alkaloid. In the plant the base exists in the *levo* form.

The presence of the quinazoline grouping in vasicine, $C_{11}H_{12}ON_2$, was shown by gentle oxidation with permanganate, which resulted in 4-keto-3,4-dihydroquinazolyl-3-acetic acid (II). In this product, $C_{10}H_8O_3N_2$, only one of the vasicine carbon atoms is missing; this

H$_2$

N——CH$_2$

C   CH$_2$

N   C   C
     H   OH
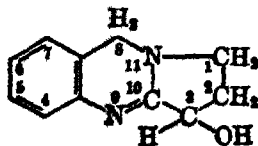
I. Vasicine (Peganine)

O
C
N·CH$_2$COOH

N·CH
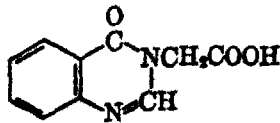
II. 4-Keto-3,4-dihydroquinazolyl-3 acetic acid

[176] Preobrashenski and co-workers, *Ber.*, **66**, 1187, 1536 (1933); **68**, 844, 847, 850 (1935); **69**, 1314, 1535 (1936).

carbon atom must carry a group that makes it especially susceptible to oxidation. It is indeed the seat of the alcoholic hydroxyl group, whose presence can be shown by gentle acetylation, by the Zerewitinoff reaction (p. 500), and by the chlorination and reduction procedure that leads to desoxyvasicine (pegene-9) (VI).

The structure of II was easily established by synthesis. For the attachment of the three-carbon chain, one end of which is certainly linked to the 11-position, only C-8 or C-10 comes into question. This uncertainty was removed by the synthesis of desoxyvasicine.[177] o-Nitrobenzyl chloride was condensed with methyl 4-aminobutyrate to the pyrrolidone (IV), which was reduced to the amino derivative (V) and treated with phosphorus oxychloride to close the quinazoline ring.
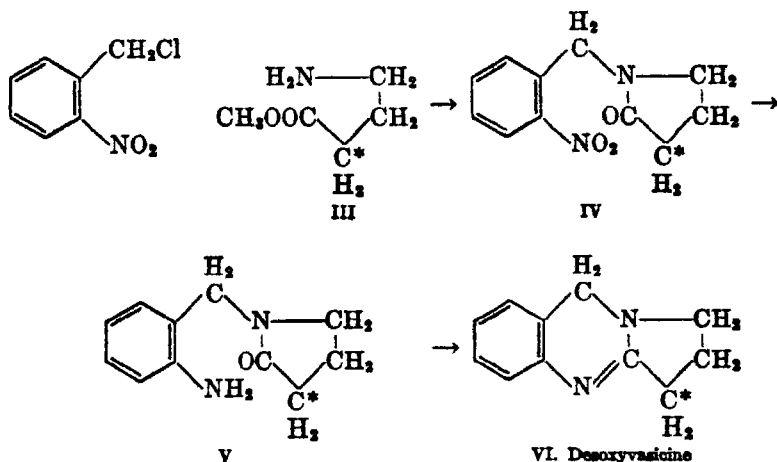
The alcoholic hydroxyl group of vasicine might be located at positions -2, -3, or -8, the first two being the more probable. The decision in favor of position -3 was reached through Späth's vasicine synthesis, which proceeded like that of desoxyvasicine (formulas III to VI, OH at the starred carbon atom). o-Nitrobenzyl chloride was condensed with 4-amino-2-hydroxybutyric acid methyl ester to the pyrrolidone. On reduction of the nitro group, spontaneous ring closure took place to give vasicine.[178]

In confirmation of considerations on the possible mode of the phytochemical synthesis of vasicine, Schöpf has prepared desoxyvasicine under physiological conditions (p. 1256).

[177] Späth, Kuffner, and Platzer, *Ber.*, **68**, 497 (1935); Hanford and Adams, *J. Am. Chem. Soc.*, **57**, 921 (1935).

[178] Späth, Kuffner, and Platzer, *Ber.*, **68**, 699 (1935).

BIOGENESIS OF THE ALKALOIDS

One of the most striking characteristics of an alkaloid-bearing plant is its capacity to produce a number of closely related bases. Examination of any of the series of alkaloids described under various nuclear groups in the preceding pages inevitably suggests that the plant, with its preëminent synthetic ability, has built up such a series from a common parent substance through condensations, methylation, decarboxylation, and oxidation and reduction reactions. This idea was first suggested early in this century by Pictet and by Willstätter, and was put into definite form by Winterstein and Trier (1910) and particularly by Robinson (1917).[179]

The amino acids (p. 1079), or their transformation products, the amino aldehydes and amines, with formaldehyde, formic acid, and methanol, undoubtedly are the chief building units for the synthesis of alkaloids. The nearer the alkaloid to the parent substance in structure, the more widely it will be found distributed in plants. Hordenine, first found in sprouted barley, probably is formed by N-methylation of tyrosine, and its appearance in the entirely unrelated *Anhalonium* cactus (anhaline, p. 1209) is not surprising. Arabine and loturine are identical with harman, the framework of the harmala alkaloids. The close relationship of harman to tryptophan or tryptamine accounts plausibly for the presence of the harman grouping in three unrelated plant families.
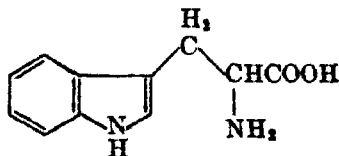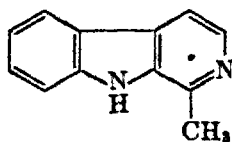
Tryptophan   Harman

Individual species or families, on the other hand, may possess a characteristic ability to cause condensations and ring enlargements leading to the synthesis of alkaloids peculiar to the species.

The first experimental demonstration of the simplicity of method by which the plant may synthesize alkaloid structures was Robinson's condensation of succinic aldehyde and methylamine with acetonedicarboxylic acid (p. 1197). This pioneering experiment stands out in sharp contrast to the involved and laborious synthesis of tropinone by the classical laboratory methods, and it furnished the stimulus for the present activity in alkaloid synthesis under approximated physiological conditions.

[179] Robinson, *J. Chem. Soc.*, **111**, 876 (1917).

According to Schöpf,[180] synthesis in the plant cell may take place with participation of specific enzyme systems, adapted to the production of one definite substance, as for example the synthesis of starch from carbon dioxide; or unspecific enzymes may take part, such enzymes as have a general function, as decarboxylation, hydrogenation, dehydrogenation, and oxidation; or finally, natural products, or the intermediates from which they are derived, may be formed without the participation of enzymes, when sufficiently reactive units arise together in the course of cell metabolism. This last case is susceptible of study in the laboratory. Essential conditions to be observed are hydrogen-ion concentrations and temperatures comparable to those under which the plant works, as well as the use of starting materials that the plant may be expected to have available.

There can be no doubt as to the ability of the plant to reduce ketone groups and to accomplish esterification, so that the question of the phytochemical synthesis of the alkaloids of the belladonna group, the tropine derivatives, is largely that of the synthesis of tropinone. Succinic aldehyde (from degradation of ornithine), methylamine, and acetonedicarboxylic acid are all cell-possible substances, but Robinson's synthesis is open to objections, because the condensation leading to tropinonedicarboxylic acid was carried out in strongly alkaline solution, and the subsequent decarboxylation required physiologically impossible conditions. When, however, the condensation is accomplished in buffered solution (0.04 molar) between pH 3 and pH 11 at 25°, spontaneous decarboxylation takes place, and tropinone is obtained in excellent yields. The same mechanism may be imagined to operate in the formation of pseudopelletierine (p. 1182), the ring homolog of tropinone, in the plant. Here glutaric aldehyde, conceivably arising in the cell from the degradation of lysine, $NH_2CH_2CH_2CH_2CH_2CH(COOH)NH_2$, takes the place of succinic aldehyde. By condensing glutaric aldehyde with methylamine and acetonedicarboxylic acid in solutions buffered to pH 7 at 25°, Schöpf was able to prepare pseudopelletierine in nearly quantitative yield.[181]

The cocaine types, derived from ecgonine, demand the retention of one carboxyl group during the condensation. This, also, can be accomplished under physiologically possible conditions (pH 5), if the monomethyl ester of acetonedicarboxylic acid is used.

$$
\begin{array}{ccc}
\text{CH}_2\text{—CHO} & \text{CH}_2\text{COOCH}_3 & \text{CH}_2\text{—CH——CHCOOCH}_3 \\
| & | & | \quad\quad | \\
\quad\quad \text{H}_2\text{NCH}_3 + \text{CO} & \rightarrow & \text{NCH}_3 \quad \text{CO} \\
| & | & | \quad\quad | \\
\text{CH}_2\text{—CHO} & \text{CH}_2\text{COOH} & \text{CH}_2\text{—CH——CH}_2
\end{array}
$$
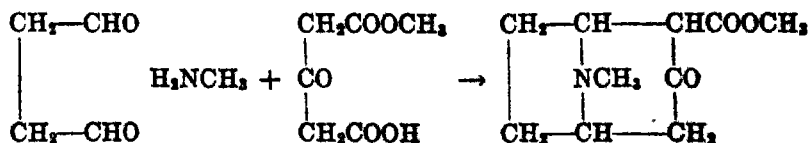
[180] Schöpf, *Ann.*, **497**, 1 (1932).
[181] Schöpf and Lehmann, *Ann.*, **518**, 1 (1935).

The alkaloids containing a quinoline nucleus could reasonably be supposed to be formed through a Friedländer synthesis, condensation of o-aminobenzaldehyde with ketones. The oxidation product of o-amino-benzaldehyde, namely anthranilic acid, is found frequently in nature, and is observed as a degradation product from tryptophan in the animal body. Methyl ketones are present in many ethereal oils. The Friedländer condensation, which would lead to the quinoline alkaloids of the angostura type, proceeds, however, only in alkaline solution, at pH 11–12. The biosynthesis of the quinoline group appears to have its starting point in substances that may be regarded as the progenitors of the methyl ketones, namely the β-keto acids. The synthesis of one of the members of the angostura alkaloids (p. 1208) illustrates this sufficiently. Condensation of very dilute solutions of o-aminobenzaldehyde and caproylacetic acid at 25° and pH 7–9 resulted in an excellent yield of α-n-amylquinoline.[182]
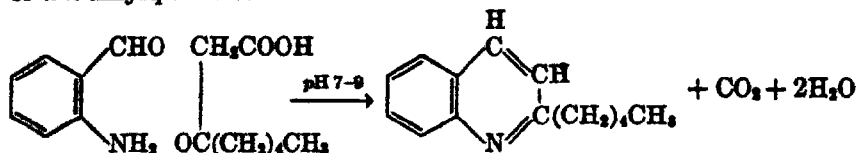
The substituted phenylethylamines have long been considered as the probable parent substances of the extensive group of isoquinoline alkaloids. Most of these alkaloids carry groups in the 5 and 6 positions and might be formed from condensation of the appropriate aldehyde with the dihydroxyphenylethylamine (I), arising from degradation of dihydroxyphenylalanine. The reaction with acetaldehyde, for example, takes place readily at ordinary temperatures in the pH range 3–5, to give the
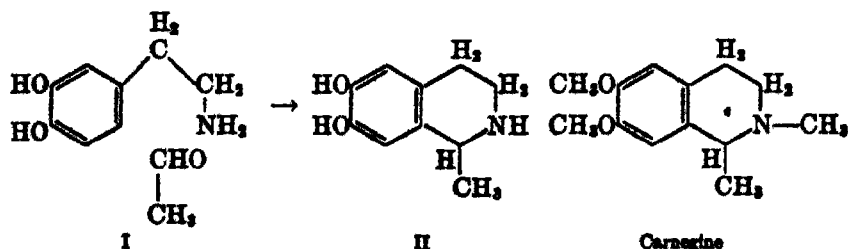
tetrahydroisoquinoline derivative (II), which is a demethylated analog of the alkaloids carnegine and salsoline.[183]

The fact that carnegine and salsoline occur naturally as the racemic forms makes it seem probable that they are synthesized in a similar way,

[182] Schöpf and Lehmann, Ann., 497, 7 (1932).
[183] Schöpf and Bayerle, Ann., 513, 190 (1934).

and not under the influence of enzymes. The condensation under physiological conditions has been extended by Hahn to the synthesis of benzylisoquinoline bases of the laudanosine type.[184]

For the ever-increasing group of alkaloids containing the indole nucleus, the building unit must be tryptamine, derived from tryptophan by decarboxylation. In this series, too, the mechanism has been subjected to direct investigation. Tryptamine reacts with acetaldehyde at ordinary temperatures, and at pH 5–7, to give tetrahydroharman.[185]
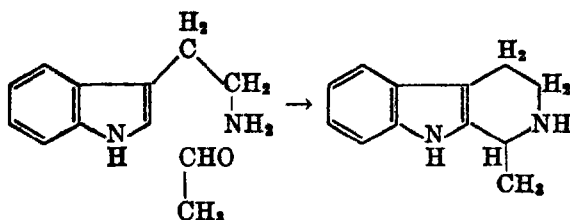
The reaction does not proceed satisfactorily with more complicated aldehydes, but succeeds with α-keto acids, which are probably the biochemical progenitors of the aldehydes. It is interesting to note that the condensation is accelerated markedly by sunlight. The problem of decarboxylation under physiological conditions of the condensation products from the α-keto acids has not been solved, but Hahn has used the method for the synthesis of the complicated skeleton present in the yohimbine group.[182]

The synthetic methods outlined above have recently been applied to the construction of another complex nucleus, that of vasicine (III). The quinazoline system present in vasicine may be imagined as arising from interaction of o-aminobenzaldehyde and α-hydroxy-γ-aminobutyralde-
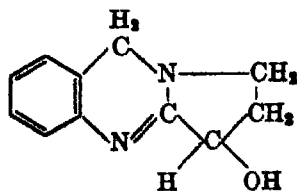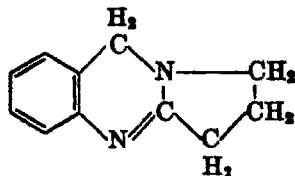
III. Vasicine   IV. Desoxyvasicine

hyde, followed by isomerization and shift of two hydrogen atoms. Unfortunately, α-hydroxy-γ-aminobutyraldehyde is not known, but γ-aminobutyraldehyde, in the form of the diethylacetal, is available, and the synthesis of desoxyvasicine (IV) by the use of this aminoaldehyde makes the above hypothesis of the biogenesis of vasicine seem reasonable.

[184] Hahn and Schales, *Ber.*, **68**, 24 (1935).
[185] Hahn and Ludewig, *Ber.*, **67**, 2031 (1934).

In dilute solution, at pH 5, γ-aminobutyraldehydediethylacetal undergoes rapid hydrolysis and the liberated aldehyde condenses with o-aminobenzaldehyde to the pseudo-base V. The pseudo-base isomerizes to the colored quaternary ammonium base VI, in which a shift of two hydrogen atoms to the quinazoline formula of desoxyvasicine takes place under the influence of palladium and hydrogen.

V. Pseudo-base

VI. Ammonium base

IV. Desoxyvasicine
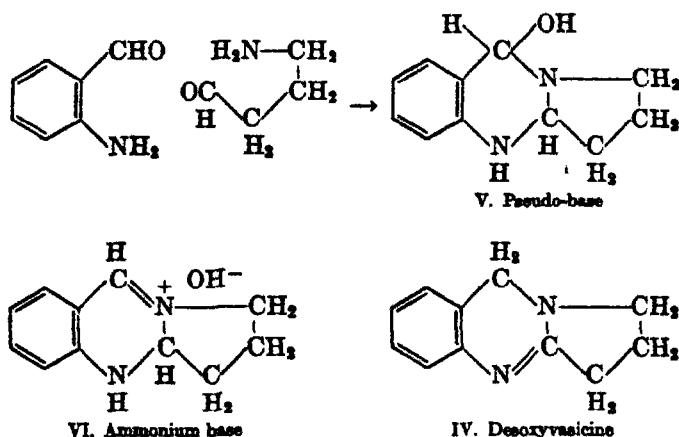
If this process represents the biosynthetic course of vasicine formation, the last step, hydrogen shift, probably takes place in the plant through the action of enzymes.[136]

Several other syntheses under similar conditions, as for example that of hygrine [137] and of lobelanine,[131] have been accomplished. Attempts to verify inviting theoretical relationships through oxidation or dehydrogenation reactions have not been successful. The conversion of hygrine to tropinone, which might be expected to proceed as follows, failed:
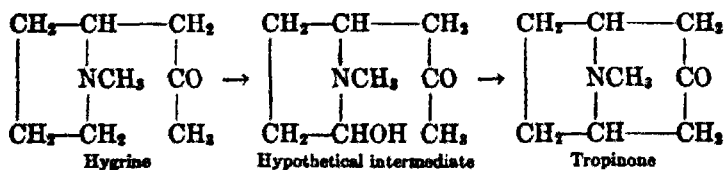
The formal relationship existing between alkaloids of the benzylisoquinoline type and those of the aporphine or morphine series, as for example laudanosine and glaucine, has led to fruitless attempts to establish the missing linkage in such types.

[136] Schöpf and Oechler, *Ann.*, **523**, 1 (1936).
[137] Robinson, *J. Chem. Soc.*, 1079 (1936).

CH₃
|
N—CH₂
H₂ H /   \ CH₂
C—C   CH₂

CH₃O

CH₃O   OCH₃   OCH₃

Laudanosine

CH₃
|
N—CH₂
H₂ H /   \ CH₂
C—C   CH₂

CH₃O

CH₃O   OCH₃   OCH₃

Glaucine
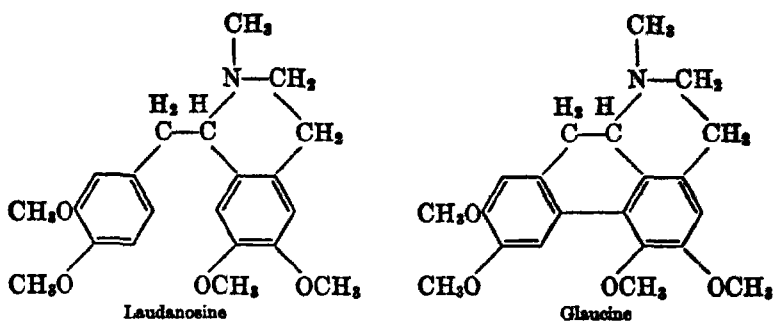
The hypothetical progenitor of the sinomenine series, protosinomenine, has been synthesized, and experiments on its conversion to sinomenine are in progress.[187]

H₂ H NCH₃
C—C CH₂
CH₂
—CH₂—OCH₃

CH₃O   OH   OH

Protosinomenine

H₂ H NCH₃
CH₂
H
CH₂ OCH₃
H₂
CH₃O   OH   O

Sinomenine
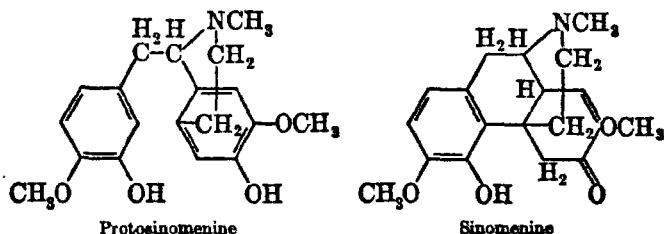
In spite of the disappointments mentioned, the success of the biosynthetic methods described is inspiring. It can be predicted that, with refinements of technique and choice of more suitable reactants, syntheses of this type can be extended to afford a great deal of additional information on the probable mechanism of formation of the alkaloids in the plant.

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CHAPTER 16

THE CHEMISTRY OF THE PORPHYRINS

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The Johns Hopkins University

CONTENTS

INTRODUCTION

Hemoglobin and chlorophyll are, perhaps, the most important pigments in the economies of the higher animals, including man. Chlorophyll acts as an intermediary in the fixation of the sun's energy in the process of photosynthesis [1] and is, consequently, responsible ultimately for all our food and most of our heat and power. The efficiency of hemoglobin as a transporter of oxygen, as well as its versatility in being also a transporter of part of the carbon dioxide formed by combustion processes in the body, make possible the efficient mechanical performance of the higher animals.[2]

Hemoglobin is a conjugated protein made up of heme, a complex of protoporphyrin and ferrous iron,[3] and globin,[4] a water-soluble, slightly basic protein, in the proportion [5] of 1 part of heme, molecular weight 616, to 26 parts of globin. It is interesting to note that no base of known structure has yet been discovered which will form a complex with heme and at the same time confer upon the heme the property of combining reversibly with oxygen in the manner of hemoglobin.[6]

Catalase [7] is another conjugated protein which contains the same heme as hemoglobin, combined with a different protein. Catalase has the property of converting hydrogen peroxide to water and oxygen, and it is presumably because of this that hydrogen peroxide bubbles when poured on a wound. It does not possess the property of combining reversibly with oxygen, as does hemoglobin, and, conversely, hemoglobin does not possess the property of destroying hydrogen peroxide except to an extremely limited degree. These two examples illustrate the point that the physiological activity of iron-porphyrin complexes is largely determined by the nature of the constituent base.[8] Chlorophyll, at least as isolated, contains no protein and has as its metallic constituent magnesium instead of iron. Its chromophoric group is also

[1] See Gaffron, "Chemical Aspects of Photosynthesis," *Ann. Rev. Biochem.*, **8**, 483–502 (1939).

[2] For a review of the literature on this subject see Peters and Van Slyke, "Quantitative Clinical Chemistry," Williams and Wilkins, Baltimore (1931), Vol. 1, Chapter XII, p. 518.

[3] Conant, *J. Biol. Chem.*, **57**, 401(1923); see also Conant and Tongberg, *ibid.*, **86**, 733 (1930).

[4] Robertson, *ibid.*, **13**, 455 (1913); for a review see Anson and Mirsky, "Hemoglobin, the Heme Pigments and Cellular Respiration," *Physiol. Rev.*, **10**, 506–46 (1930).

[5] Cohn, Hendry, and Prentiss, *J. Biol. Chem.*, **63**, 721 (1925).

[6] See W. M. Clark, "Potentiometric and Spectrophotometric Studies of Metalloporphyrins in Coordination with Nitrogenous Bases," *Cold Spring Harbor Symposia on Quantitative Biology*, **7**, 18 (1939).

[7] Sumner and Dounce, *J. Biol. Chem.*, **127**, 439–47 (1939).

[8] Barron, *Physiol. Rev.*, **19**, 184 (1939); Warburg, *Ergeb. Enzymforsch.*, **7**, 240–43 (1938).

modified from a porphyrin to a chlorin, as will be detailed in the succeeding chapter, resulting in the green color characteristic of leaves.

Fortunately, the chemistry of the porphyrin constituents of these complexes is now well understood, owing mainly to the researches of W. Küster, R. Willstätter, and Hans Fischer and their collaborators. The first part of this chapter will be devoted to recounting the organic chemical work which led to the elucidation of the structure of hemin, the ferric iron-porphyrin complex obtained in crystalline form when hemoglobin is broken down in the presence of sodium chloride and acetic acid.[9]

SURVEY OF THE DEGRADATIONS OF HEMIN

Hemin was first crystallized, under the miscroscope, by Teichmann in 1853.[10] Hoppe-Seyler undertook large-scale preparation of the material for chemical study in 1868.[11] He prepared the first porphyrin in 1871 [12] and by his degradations at that time inferred the relationship of hemin to pyrrole.[13] The complete synthesis of hemin was described by Fischer and Zeile in 1929.[14] The structure finally established by this synthesis is as follows: *
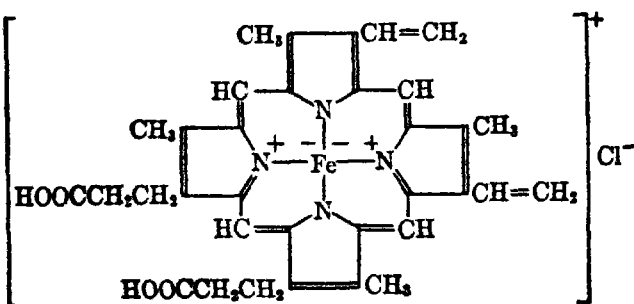
FIG. 1.—Hemin.

[9] Schalfejeff, J. Russ. Chem. Soc., 30 (1885); Nencki and Zaleski, Z. physiol. Chem., 30, 390 (1900); see also Fischer-Orth, "Die Chemie des Pyrrols," Akad. Verlag., Leipzig (1937), Bd. II, 1 Hälfte, p. 377.

[10] Teichmann, Z. ration. Med., N.F. 3, 375 (1853).

[11] Hoppe-Seyler, Med. Chem. Untersuchungen, 3, 379 (1868).

[12] Ibid., 4, 540 (1871).

[13] Ibid., 4, 524 (1871).

[14] Fischer and Zeile, Ann., 468, 114 (1929).

* The distribution of the double bonds in the formula, which must be regarded as a conventionalized abbreviation of a complex resonating system, will be discussed later. The selection of two bonds as semi-polar is an arbitrary convention. It is even possible that the chlorine is not ionised, as represented, but resembles more closely the binding in ferric chloride. In this formula and all others which follow, the four carbons of the pyrrole rings will be represented by four of the corners of a pentagon but the nitrogen will be written in.

The burden of this discussion will be the analytical and synthetic evidence which has established the carbon-nitrogen structure formulated above.

In 1896 Küster reported the isolation of hematinic acid,[15] $C_8H_9O_4N$, also called biliverdinic acid, from the products of the chromic acid oxidation of hemin. The proof of the structure of this compound is based upon that of methylethylmaleimide, which will be presented next.

When hemin is reduced mildly and the iron removed, a substance known as mesoporphyrin is obtained.[16] On oxidation of mesoporphyrin with chromic acid a new product appears together with the hematinic acid. This is methylethylmaleimide, I. Küster synthesized this material by the method shown in Fig. 2.[17]

$$CH_3COCH_2COOC_2H_5 + NaOC_2H_5 \rightarrow Na^+(CH_3COCHCOOC_2H_5)^- + C_2H_5I \rightarrow$$

$$
\begin{array}{c}
CH_3COCHC_2H_5 \\
| \\
COOC_2H_5
\end{array}
+ HCN \rightarrow
\begin{array}{c}
CH_3C(OH)CHC_2H_5 \\
| \quad\quad | \\
CN \quad COOC_2H_5
\end{array}
+ H_2O \xrightarrow{HCl}
$$

$$
\begin{array}{c}
CH_3C(OH)CHC_2H_5 \\
| \quad\quad | \\
HOOC \quad COOH
\end{array}
\xrightarrow{Heat}
\begin{array}{c}
CH_3C{=\!=}CC_2H_5 \\
| \quad | \\
O{=}C \quad C{=}O \\
\backslash\; /\\
O
\end{array}
+ NH_3 \rightarrow
\begin{array}{c}
CH_3C{=\!=}CC_2H_5 \\
| \quad | \\
O{=}C \quad C{=}O \\
\backslash\; /\\
NH
\end{array}
$$

I
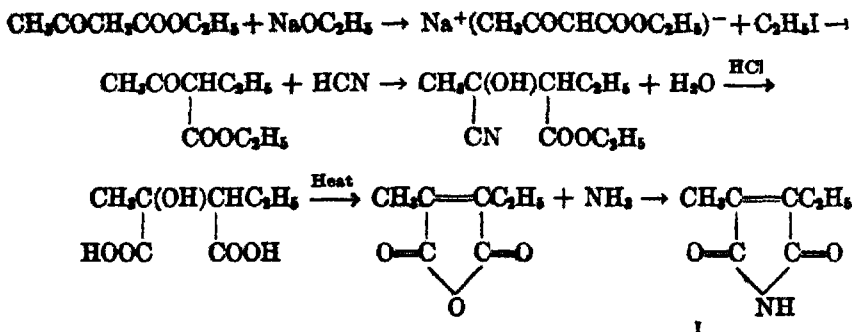
<div align="center">FIG. 2</div>

The fact that methylethylmaleimide appears only after mild reduction suggests that unsaturated groups reducible to ethyl groups are present in the hemin molecule and are destroyed in the direct oxidation.

The structure of hematinic acid was inferred from its degradations.[18a] Thus its decarboxylation gave methylethylmaleimide. This reaction left three possible structures for hematinic acid:

$$
C_8H_9O_4N \rightarrow CO_2 +
\begin{array}{c}
CH_3C{=\!=}CC_2H_5 \\
| \quad | \\
O{=}C \quad C{=}O \\
\backslash\; /\\
NH
\end{array}
$$

I
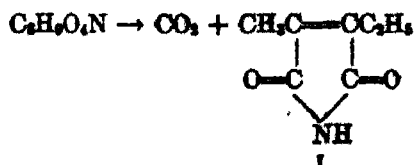
[15] Küster, Ber., **29**, 823 (1896).
[16] Nencki and Zaleski, Ber., **34**, 996 (1901); Zaleski, Z. physiol. Chem., **37**, 57 (1902).
[17] Küster, Ann., **345**, 1 (1906); see also Fittig and Parker, Ann., **267**, 204 (1880).
[18] (a) Degradations of hematinic acid: Küster, Ann., **315**, 174 (1900); see also ref. 17 (b) Synthesis: Küster and Weiler, Ber., **47**, 533 (1914).

$C_4H_9O_4N$ may be
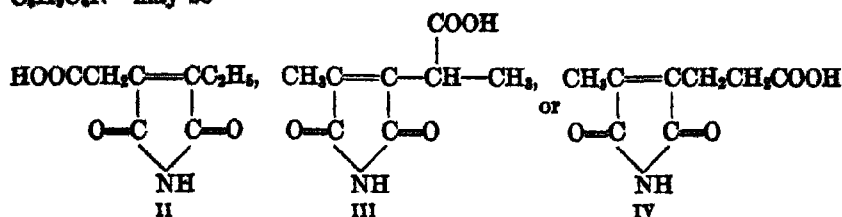
FIG. 3

Upon oxidation, hematinic acid yielded succinic acid. This excluded formulas II and III and presented a reasonable formula IV as a basis for further structural deductions. Hematinic acid has been synthesized [18b] by a series of reactions strictly analogous to those cited above for methylethylmaleimide.

Nencki [19] discovered that hydrogen iodide was capable of cleaving hemin to give a mixture of pyrrole derivatives. This mixture was worked over by Nencki, Willstätter, Küster, Knorr, Marchlewski, Piloty, H. Fischer, and others, and, in all, [20] eight pyrrole derivatives were isolated. These were:
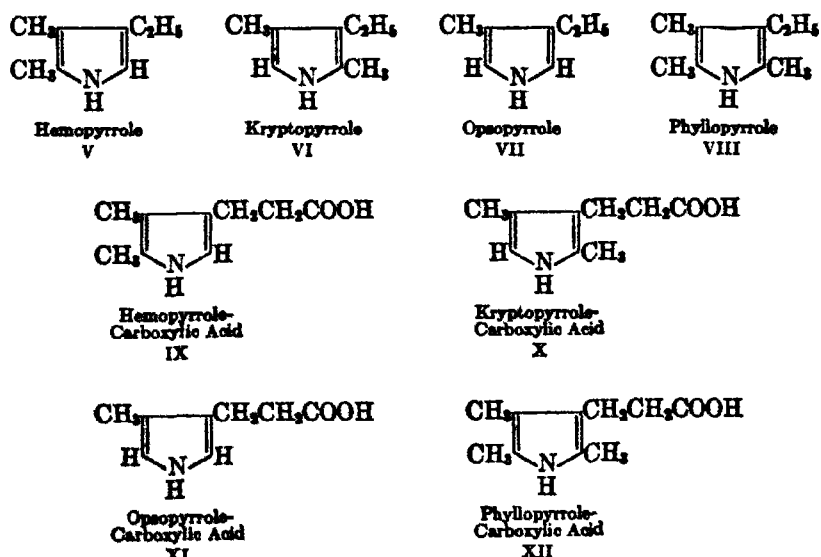
FIG. 4

[19] Nencki and Sieber, *Arch. exptl. Path. Pharm.*, 18, 418 (1884); Nencki and Zaleski, *Ber.*, 34, 1002 (1901).

[20] For a summation of the literature on the hemopyrroles up to 1914 see Piloty, Stock, and Dormann, *Ann.*, 406, 342 (1914)

THE STRUCTURES OF THE BASIC CLEAVAGE PRODUCTS OF HEMIN

Modern pyrrole syntheses of value in establishing the structure of the blood pigment stem from the studies made on the condensations of acetoacetic ester by Knorr in 1886.[21] Knorr found that isonitroso-acetoacetic ester could be reduced and condensed with acetoacetic ester in the same reaction mixture. In 1910[22] Piloty discovered that the reduction product could be isolated in the form of its hydrochloride and then condensed later by slow neutralization in the presence of acetoacetic ester. These reactions are sketched below:

$$CH_3COCH_2COOC_2H_5 + HNO_2 \rightarrow$$

$$CH_3COCCOOC_2H_5 + Zn + HCl \rightarrow CH_3COCHCOOC_2H_5$$
$$\underset{NOH}{\|} \qquad\qquad\qquad \underset{NH_3{}^+Cl^-}{|}$$
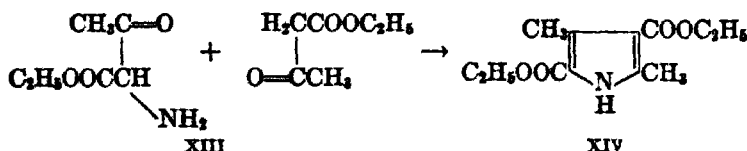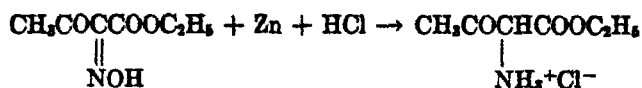
Fig. 5

Pyrrole XIV was shown by analysis to have the composition $C_{12}H_{17}O_4N$.[21] Hydrolysis gave a dibasic acid, $C_8H_9O_4N$, XV, showing the loss of two ethyl groups. This acid, on heating, readily lost two moles of carbon dioxide, giving a pyrrole derivative, C_6H_9N, XVI. On oxidation with chromic acid[23] this gave citraconic imide, XVII, which had been synthesized much earlier.[24] The degradations of pyrrole XIV are formulated below:
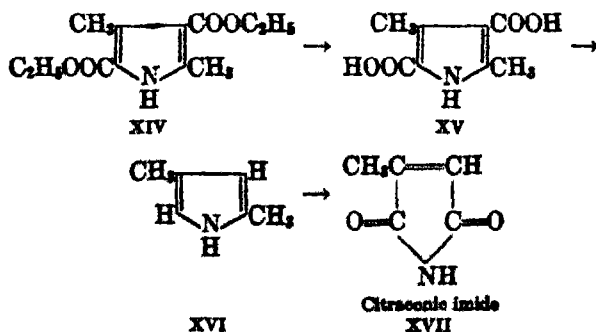
Fig. 6

[21] Knorr, Ann., 236, 317 (1886).
[22] Piloty, Ber., 43, 489 (1910).
[23] Plancher and Cattadori, Gazz. chim. ital., 33 I, 405 (1903).
[24] Ciamician and Dennstedt, ibid., 12, 500 (1882).

v

From the information given above it will be seen that the structure of Knorr's pyrrole, XIV, as a derivative of 2,4-dimethylpyrrole is established. The synthesis excludes 2,3- as a possibility. The oxidation excludes 3,4- as well as 2,5- or any ethylpyrrole derivative.

Although some of the structures involved had been established earlier by independent methods, perhaps the most elegant method used to establish the constitution of the basic cleavage products of hemin was based on a discovery made by Fischer and Bartholomäus [25] in 1912 that pyrroles could be alkylated by autoclave reactions with sodium alcoholates. This reaction goes preferentially in the α-position but may be forced in the β-position by raising the temperature. The interrelationships worked out by Fischer are given below:
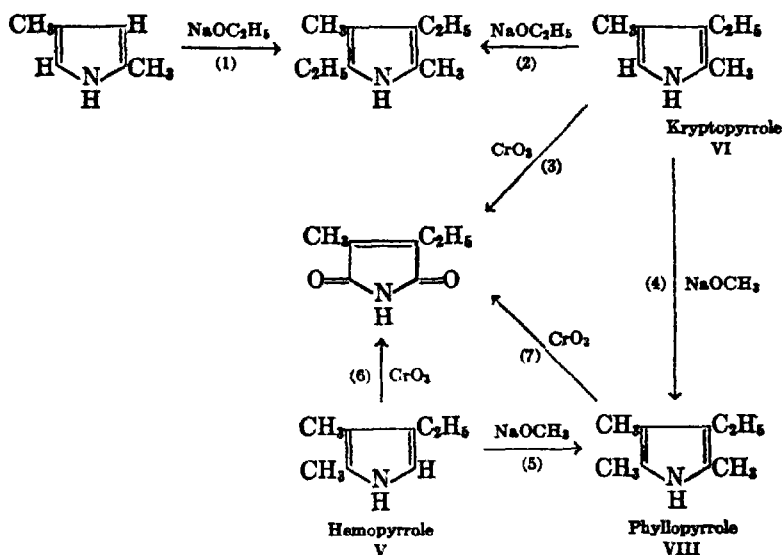
FIG. 7

Reactions (1) and (2) show that kryptopyrrole is a derivative of 2,4-dimethylpyrrole. Reaction (3) shows that it is the 3-ethyl derivative, not the 5-ethyl derivative. Reaction (4) shows that phyllopyrrole has the free 5-position of kryptopyrrole filled by a methyl group. Reaction (5) shows that hemopyrrole is directly related to phyllopyrrole and must therefore be the 4,5-dimethyl-3-ethyl (2,3-dimethyl-4-ethyl) derivative of pyrrole. Reactions (6) and (7) confirm the presence of β-methyl and ethyl groups.

²⁵ Fischer and Bartholomäus, Ber., 45, 466 (1912).

As an example of the type of synthesis used in preparing these substances we may give the preparation of kryptopyrrole. This was synthesized from acetylacetone, XVIII, by the following series of reactions:

$$CH_3C{=}O \quad + \quad H_3CCOCH_3 \quad \rightarrow$$
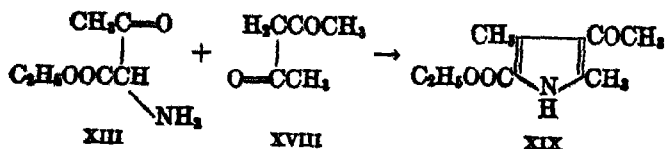
FIG. 8

The acetylpyrrole so obtained, XIX,[26] was subjected to the Wolff-Kishner method of reduction, which is specific for carbonyl groups.[26, 27] The 5-carbethoxy group was hydrolyzed and decarboxylated by the excess alcoholate necessary for the reaction.
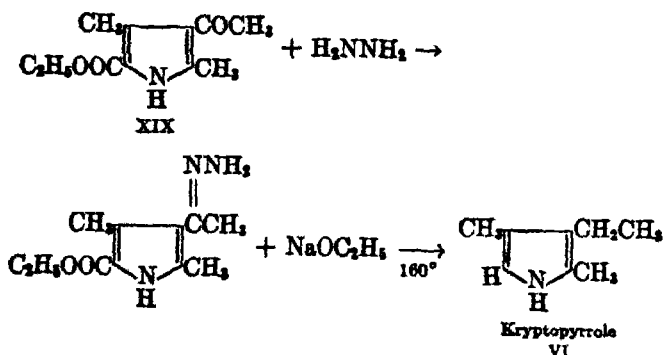
FIG. 9

THE STRUCTURES OF THE ACIDIC CLEAVAGE PRODUCTS OF HEMIN

The structures of the corresponding pyrrole carboxylic acids IX, X, XI, and XII were proved by oxidation to hematinic acid, IV, and by decarboxylation to the corresponding pyrroles,[28] whose structures had been determined previously. In the course of the succeeding decade, independent syntheses which fully confirmed the structures assigned were worked out for all these substances. In the development which follows a few typical syntheses of these compounds will be sketched as the need arises for the use of the substances in succeeding condensations.

[26] Knorr and Hess, Ber., 44, 2758 (1911).

[27] Fischer, Baumann, and Riedl, Ann., 475, 239 (1929).

[28] Piloty, Stock, and Dormann, Ann., 406, 345 (1914).

THE STRUCTURES OF DIPYRRYLMETHENES

In 1915 Fischer[29] showed that the bromination of kryptopyrrole or of hemopyrrole gave dipyrrylmethenes, and in 1926[30] Fischer and Klarer found that these gave porphyrins on heating with acid. Since most porphyrins are made from dipyrrylmethenes, the structures of the methenes must be established beyond doubt. The product obtained by the bromination of kryptopyrrole is chosen as an example, since considerable effort has been expended to establish its structure.

A careful investigation of the substance obtained by the bromination of kryptopyrrole showed that it was a mixture of at least two compounds:[31]
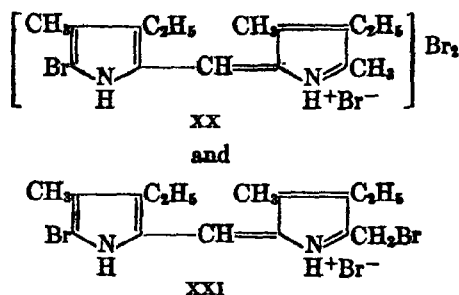
FIG. 10

Either of these substances on treatment with acid yielded a porphyrin. When the extra bromine of the perbromide XX was removed, however, the resulting methene
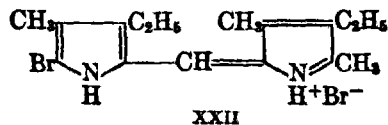
FIG. 11

was not capable of giving porphyrin upon identical treatment. This suggested that the ability of methene XX to give porphyrin was conditional upon its ability to be further brominated by its perbromide bromine to methene XXI. It should be noted that the position of the extra bromine in compound XXI has never been established unequivocally, but for the sake of general consistency with known pyrrole reactions and with the absence of bromine in the porphyrins formed from

[29] Fischer, *Sitzber. math. naturw. Abt. bayer. Akad. Wiss. München*, 412 (1915).

[30] Fischer and Klarer, *Ann.*, 448, 186 (1926).

[31] Fischer, Baumann, and Riedl, *Ann.*, 475, 216–236 (1929); also Fischer and Kirrmann, *Ann.*, 475, 277 (1929).

this and similar methenes it is formulated as being upon the α-methyl group. Methene XXII can be brominated to XXI, and the extra bromine can be removed by mild treatment with hydrogen iodide. Since this interconversion is possible, we shall concern ourselves only with the proof of the structure of methene XXII.

Initially,[29] the formulation of methene XXII was assigned because of the products obtained upon reductive cleavage of the material.
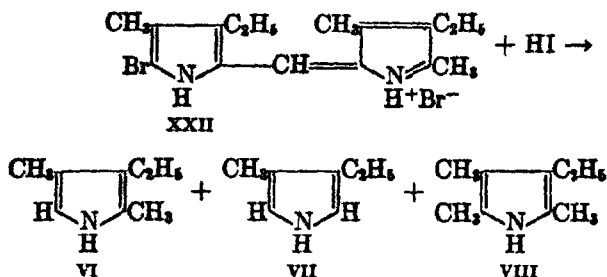
FIG. 12

Because the carbon which was originally present in the 2-position of kryptopyrrole turned up in the 5-position of the phyllopyrrole obtained as a cleavage product, it was inferred that it occupied both positions in the condensation product and hence was present as a bridge. When the structure of this compound became of critical importance, owing to the fact that it was an intermediate in the first successful porphyrin synthesis, the formulation was reinvestigated synthetically.[31] The preparation starts from kryptopyrrole:
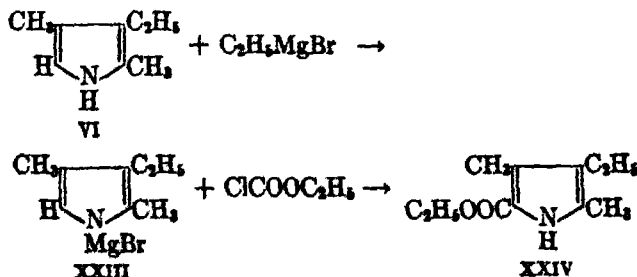
FIG. 13

A certain amount of the N—COOC₂H₅ compound, which is an oil, was formed by this reaction.[32] The preparation of the crystalline solid, XXIV, by direct hydrogenation of the acetylpyrrole, compound XIX,[33] shows that it is really the α-carbethoxy compound. This is now the

[29] Fischer and Klarer, *Ann.*, **450**, 181 (1926); Fischer, Kirstahler, and Zychlinsky, *Ann.*, **500**, 10 (1933).
[30] (a) Signaigo and Adkins, *J. Am. Chem. Soc.*, **58**, 710 (1936). (b) Corwin and Quattle-baum, *ibid.*, **58**, 1084 (1936).

method of choice in the preparation of carbethoxykryptopyrrole. The synthesis continued: [34]
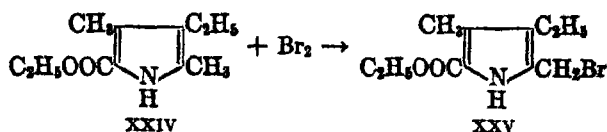
Fig. 14

Further oxidation of the 2-methyl group in XXV to the 2-carboxylic acid,[35] which can be hydrolyzed and decarboxylated to opsopyrrole, compound VII, establishes the fact that halogenation does not attack the β-positions.
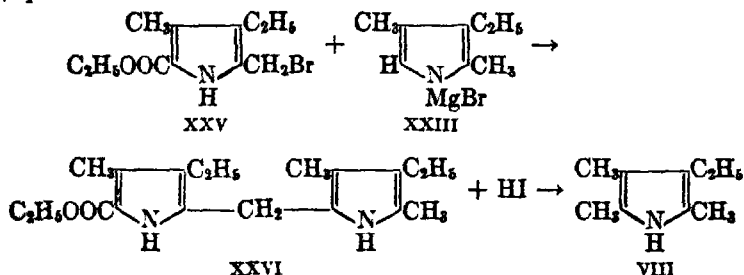
Fig. 15

To show that the entering group went into the 5-position of XXIII, not on the nitrogen, the dipyrrylmethane XXVI was reduced with hydrogen iodide to give phyllopyrrole, VIII.[31] Since the carbon atom which was known to be attached to ring I was then found attached to ring II, the dipyrrylmethane formulation was established. The synthesis then proceeded:
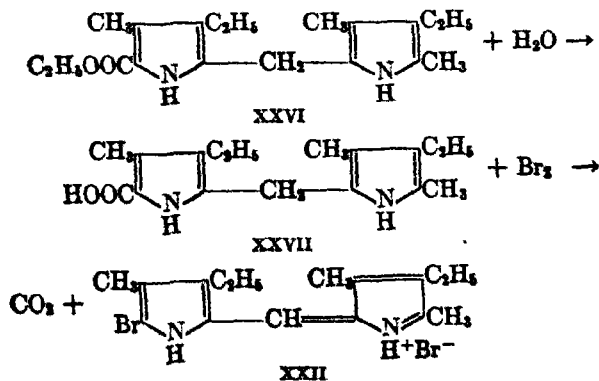
Fig. 16

[34] Fischer and Ernst, Ann., 447, 159 (1926).
[35] Fischer, Sturm, and Friedrich, Ann., 461, 269 (1928).

It should be noted that the last step in this series includes three reactions: oxidation, decarboxylation, and bromination, and that the methane-carboxylic acid XXVII has not been satisfactorily characterized by analysis. Hence there is a jump of four steps from the last well-established compound, XXVI, to the desired methene, XXII. Further substantiation of the formulation given was obtained by still another method of synthesis:
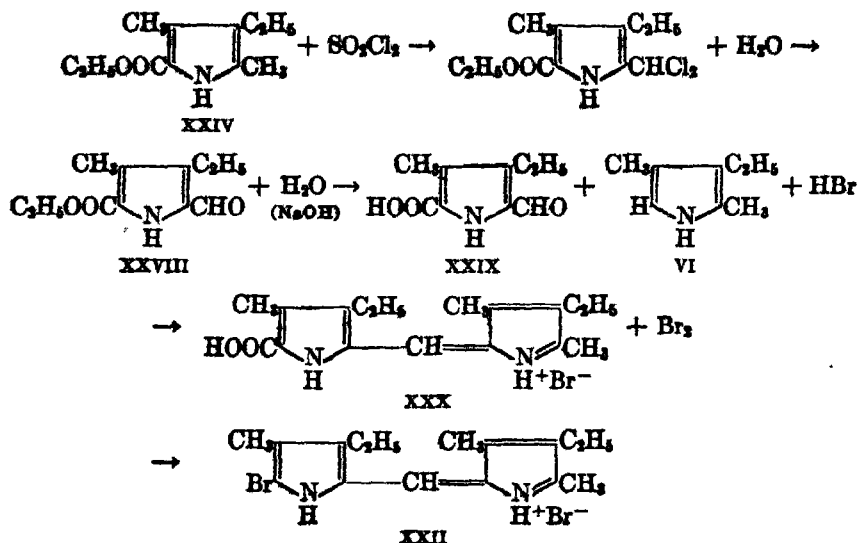
Fig. 17

It will be observed that the final step in this synthesis is a two-step reaction involving both decarboxylation and bromination. As in the preceding synthesis, neither of the two carboxylic acids XXIX or XXX [35, 31] proved amenable to satisfactory analytical characterization, so that again it is four steps from the last completely characterized reagents to the final methene. These syntheses do, however, make it clear that the identical methene system can be formed from three sets of initial products by three different routes.

THE NATURE OF THE PORPHYRIN NUCLEUS

Early Evidence

The next step in the logical development of the structure of hemin is to establish the nature of the porphyrin nucleus. With the structures of the basic and acidic cleavage products of hemin understood, we see

that the essential points in the structures of the side chains of hemin are known. A molecular-weight determination,[36] which shows that there must be four pyrrole residues in a porphyrin, combined with the results of analysis, which show that there are two carboxyl groups,[16] provide a basis for projecting possible formulas for hemin. It seems logical to suggest that the pyrrole rings are joined by bridges consisting of a single carbon atom, since all possible combinations of zero and one carbon occur in the α-positions of the pyrrole derivatives isolated, but no two-carbon residue is found.

In 1912[37] Küster advanced a formula for hemin nearly identical with that shown in Fig. 1. Shortly after this Willstätter,[38] Fischer,[39] and Piloty[20] made alternative proposals for the structure of the porphyrin nucleus. Willstätter's tetrapyrrylethylene formulation achieved considerable notice and was not disproved until 1925 when Fischer[40] prepared such a compound. Fischer's proposal was an "indigoid" formula,[40] essentially that of Willstätter but with pairs of pyrrole rings further paired by extra carbon bridges. The indigoid formula for porphyrins could hardly be entertained as a reasonable possibility, even in the absence of experimental refutation, since a model of the compound would involve strains of a sort which have never been observed in organic compounds. On the basis of constantly accumulating synthetic evidence[41] all these suggestions were finally discarded in favor of the Küster type of formula.

Modern Evidence

Before proceeding to the details of the reasoning involved, it may be well to point out the fundamental difference between structural proofs in the porphyrin series and those in other branches of organic chemistry. The normal course of a structural proof is to study the compound in question by a number of degradation reactions sufficient to establish its structure with a reasonable degree of certainty and then to undertake a synthesis. Synthetic methods may then be used to distinguish between a small number of alternative formulations which can-

[36] Zaleski, Z. physiol. Chem., 37, 73 (1902); ibid., 43, 15 (1904); Fischer and Hahn, Ber. 46, 2308 (1913).

[37] Küster, Z. physiol. Chem., 82, 463 (1912).

[38] Willstätter and Stoll, "Untersuchungen über Chlorophyll," Julius Springer, Berlin (1913), p. 42. See also Willstätter and M. Fischer, Ann., 400, 182 (1913).

[39] Fischer and Röse, Z. physiol. Chem., 88, 263 (1913); Fischer and Klarer, Ann., 448, 183 (1926).

[40] Fischer and Beller, Ann., 444, 238 (1925).

[41] Fischer, Halbig, and Walach, Ann., 452, 268 (1927).

not be distinguished analytically. In the porphyrin field this method broke down because degradations drastic enough to break the porphyrin nucleus were also drastic enough to cleave the products of the porphyrin fission, leaving only small fragments. The method of degradation was not capable of establishing finally the nature of the porphyrin nucleus itself, much less of giving information concerning the relative positions of the side chains on the nucleus. For this reason the synthetic method was resorted to and exploited systematically by Fischer in the attack on the problem of porphyrin structure.

It should be emphasized that, in this field, perhaps more than in any other, a brief review does the logic of the structure which has been erected less than justice. This is true because one of the essential features of the logic is the general consistency of a large number of syntheses. Many of the key substances have been prepared by more than one method. Several substances in one series of compounds have been converted to those in another series which have been synthesized independently. Thus the whole porphyrin structural argument should be regarded not as a chain of links but as a fabric of interlinking chains, the number of whose interconnections probably constitutes the most convincing argument for the soundness of its parts.

The most striking physical characteristic of a porphyrin is its absorption spectrum.[11] Although porphyrins which are not isomeric may have sensibly identical spectra,[42] and although variations in the nature of the side chains of the nucleus may produce marked changes in the positions of the absorption bands and even inversions in their intensities, nevertheless, through wide variations in structure, there remains a qualitatively characteristic four-banded spectrum.

Using the absorption spectrum as the major criterion of success or failure, Fischer was able, finally, to achieve a porphyrin synthesis.[10] After this he rapidly devised alternative methods for producing the porphyrin nucleus, which showed the nature of the linkages in the ring system.

Octamethylporphyrin [43] was prepared by two methods: [44]

[41] Fischer, *Z. physiol. Chem.*, **138**, 307 (1924).

[42] Fischer and Walach, *Ann.*, **450**, 165 (1926). For the purpose of systematic nomenclature Fischer coined the term "porphin" to indicate the porphyrin nucleus. It is the opinion of the present author that this gives rise to unnecessary complications and is counter to established chemical usage. Hoppe-Seyler, who discovered the porphyrins, should be permitted to retain the honor of naming the class, in accordance with custom. It should be noted, further, that it has never been considered necessary to alter such terms as benzene, naphthalene, and anthracene when systematizing substitutions in the nuclei, e.g., 1,4-dimethylnaphthalene is not called "1,4-dimethylnaphthene."

[43] Fischer and Walach, *Ann.*, **450**, 174 (1926); Fischer, Halbig, and Walach, *Ann.*, **452**, 279 (1927).
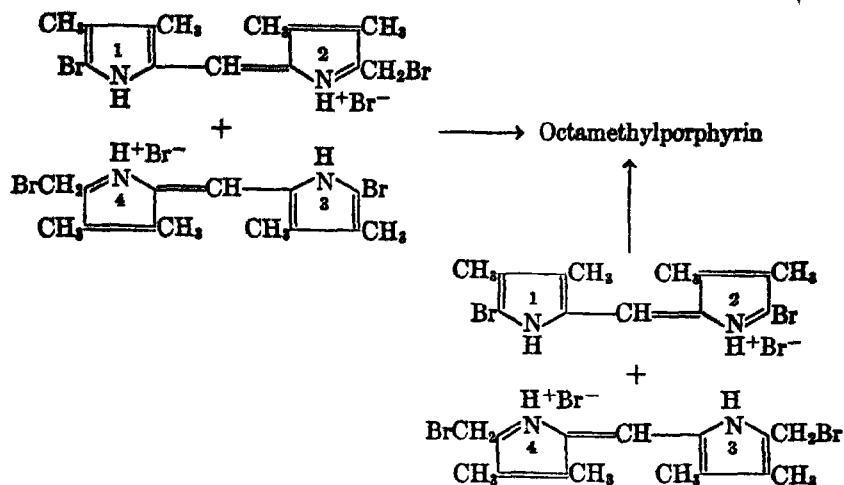
FIG. 18

If we assume that no molecular rearrangement has taken place, the CH_2Br- group on ring 2 in the first synthesis has the same function as that on ring 3 in the second synthesis. This can be true only if the carbon concerned is functioning as a bridge between rings 2 and 3 in the porphyrin. If we assume that reaction conditions favorable to the condensation of rings 2 and 3 should also favor the similar condensation of rings 1 and 4, we have grounds for assuming that the porphyrin formed is actually a large "doughnut" ring, with four equivalent carbon bridges joining four pyrrole rings.

When the syntheses above were completed, Fischer adopted the large ring formulation of Küster for the porphyrin nucleus and proceeded with numerous syntheses on this basis, none of which gave results incompatible with this formulation. A number of syntheses were also tried in which one or more of the structural elements necessary for the large ring formulation were lacking and in nearly all cases the results were negative.[45] These negative results cannot be accepted as conclusive, however, for certain syntheses in which all necessary structural elements are present also fail to go. This is due perhaps to insufficient activation of the α-positions by the groups occupying the β-positions. The fact that syntheses lacking some of the structural elements were not uniformly unsuccessful would be more disturbing were it not for the difficulty of insuring purity of the materials entering into the reaction to an extent compatible with the extremely small yields obtained. For a time the logical inconsistency involved here could be overlooked because of the general consistency obtained in a constantly increasing

[45] Fischer, Sturm, and Friedrich, *Ann.*, **461**, 254-6, 262 (1928).

number of porphyrin syntheses. Fortunately, however, Fischer later worked out a logically convincing proof of the cyclic nature of the porphyrin nucleus which, so far as we are aware, has no parallel in structural chemistry. Because of its unique nature, this proof deserves a distinctive name, and we shall call it the method of "progressive pairing of quadrants."

Examples of the method of progressive pairing of quadrants appear in the syntheses of two of the chlorophyll porphyrins, pyrroporphyrin [46a] and rhodoporphyrin.[46b] We shall choose the syntheses of rhodoporphyrin to illustrate the method and shall leave for a later section the nature of the reasoning which led to the selection of this particular order of substituents.

If we consider, geometrically, a cyclic system made up of four different structural elements, all joined, we shall see that there are two methods of putting the structure together from paired elements:
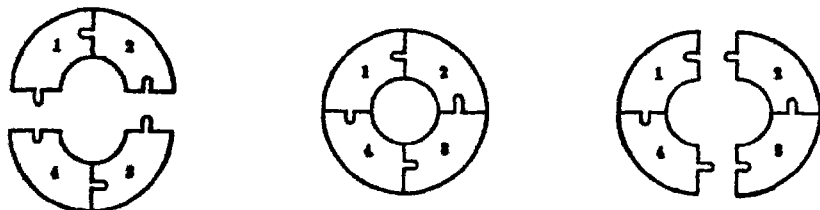
FIG. 19

A brief consideration of the geometry of the figure will show that a structure made from the pairs 1-2 and 3-4 which is identical with a structure made from the pairs 1-4 and 2-3 must be cyclic.

For the argument to retain its validity, it is not necessary that all the structural elements be different. The argument is equally valid in the following case:
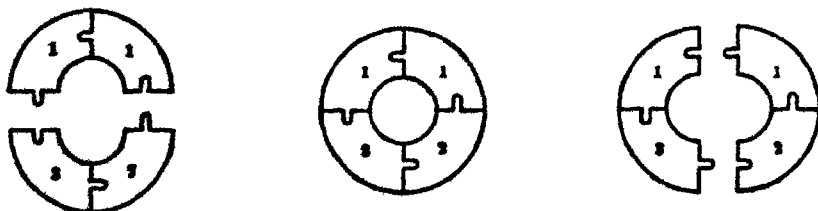
FIG. 20

It is because the argument breaks down when the four structural elements are made up of only two different kinds that it was necessary to wait until more complicated porphyrins were synthesized before the reasoning could be applied.

[46] (a) Fischer, Berg, and Schormüller, *Ann.*, **480**, 126, 144 (1930). (b) Fischer, Berg, and Schormüller, *Ann.*, **480**, 121, 134 (1930).

The first synthesis of rhodoporphyrin was accomplished in the following manner:
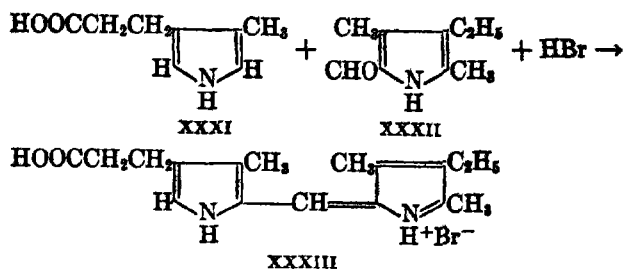
Fɪɢ. 21

The fact that the aldehyde synthesis took place by condensation under the methyl group of **XXXI** and not under the propionic acid group was demonstrated by a reversed synthesis: [47]
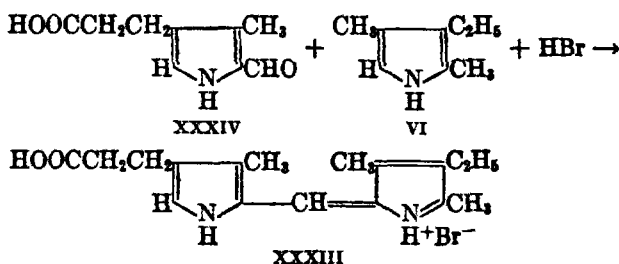
Fɪɢ. 22

For the reasoning to be convincing, it is necessary to locate the aldehyde group in compound **XXXIV**. This was prepared by the following reaction: [48]
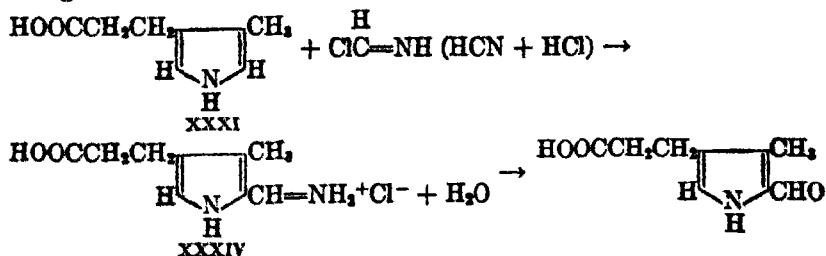
Fɪɢ. 23

Its structure was then proved by its reduction to hemopyrrole carboxylic acid,[49] whose structure was known.[28]

[47] Fischer, Berg, and Schormüller, *Ann.*, **480**, 136 (1930); Fischer, Friedrich, Lamatsch, and Morgenroth, *Ann.*, **466**, 175 (1928).
[48] Fischer and Treibs, *Ber.*, **60**, 379 (1927).
[49] Fischer and Nüssler, *Ann.*, **491**, 186 (1931).

HOOCCH₂CH₂————CH₃   H••CCH₂CH₂————CH₃
          H   N   CHO   →   H   N   CH₃
                  H   H
          XXXIV   IX
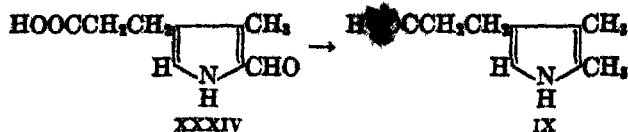
FIG. 24

This last reaction finally established the position of the linkage in the methene XXXIII.

The other methene for the synthesis of rhodoporphyrin was prepared by the following reaction:

C₂H₅OOC————CH₃   C₂H₅————CH₃
    CH₃   N   H   +   CHO   N   COOH   + HBr →
           H   H
         XXXV   XXIX

C₂H₅OOC————CH₃   C₂H₅————CH₃
    CH₃   N   —CH=   N   COOH
           H   H⁺Br⁻
              XXXVI
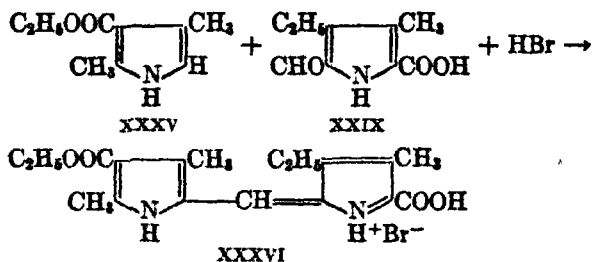
FIG. 25

Both the methenes were then brominated, the latter with decarboxylation, and the condensation to the porphyrin was brought about in an acid melt:

HOOCCH₂CH₂————CH₃   CH₃————C₂H₅
    Br   N   —CH=   N   CH₃
           H   XXXIII   H⁺Br⁻

                +   →

         H⁺Br⁻   H
    CH₃=N   —CH—   N   Br
C₂H₅OOC————CH₃   C₂H₅————CH₃
              XXXVI

HOOCCH₂CH₂————CH₃   CH₃————C₂H₅
         N   —CH=   N
         H
    HC   CH
         N   —CH=   N
              H
C₂H₅OOC————CH₃   C₂H₅————CH₃
         Rhodoporphyrin Monomethyl Ester
                   XXXVII
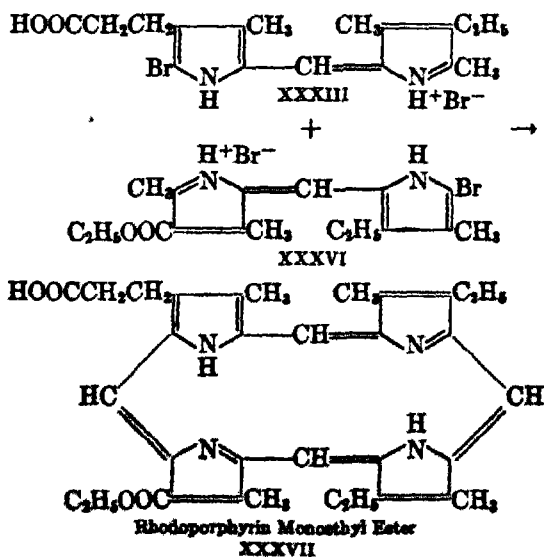
FIG. 26

The yield in this reaction was 0.1 per cent.

The alternative synthesis of rhodoporphyrin was undertaken in the following manner:
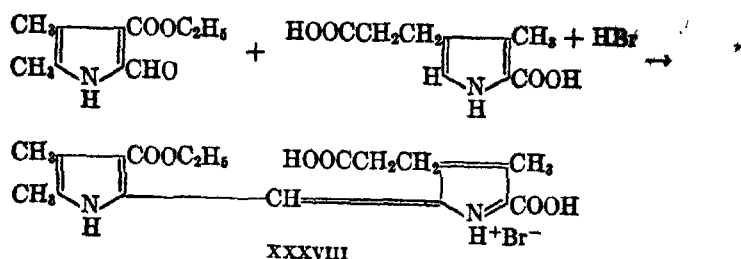
XXXVIII

FIG. 27

Methene XXXVIII lost carbon dioxide spontaneously upon recrystallization to yield the substance with a free α-position, XXXIX, which was then brominated:

XXXIX

XL
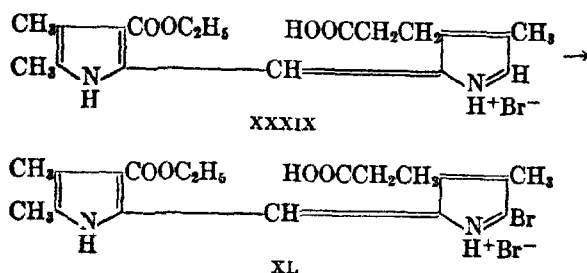
FIG. 28

The methene for the other half of the molecule was obtained by the bromination of hemopyrrole: [29]

XLI
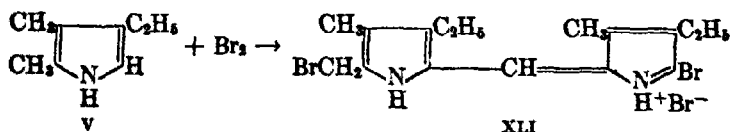
FIG. 29

These methenes were then combined in the porphyrin synthesis shown below in Fig. 30. In this synthesis, although 2 grams of the methene mixture was used, the yield was so small that only enough material for melting-point comparison was obtained. With two such examples of progressive pairing of quadrants,[46] the nature of the porphyrin nucleus was established.
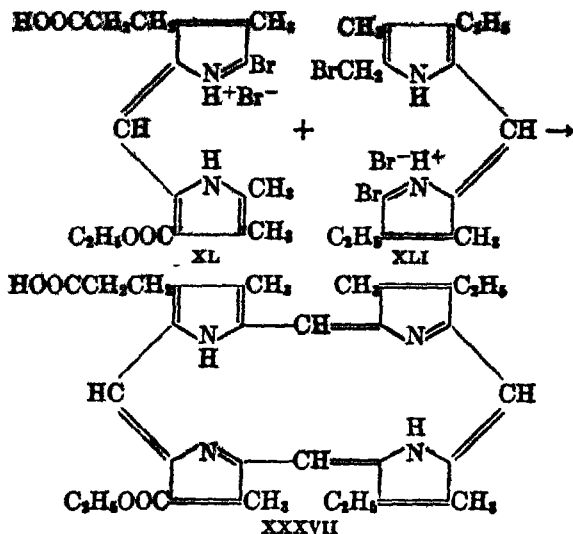
Fig. 30

CONFIGURATIONAL STUDIES

The next step in establishing the formula for hemin was to learn the order of the substituents in the porphyrin nucleus. It had been discovered that it was possible to degrade hemin, by reduction and subsequent pyrolysis, to a material called etioporphyrin.[50] Since this etioporphyrin gave methylethylmaleimide on degradation and since its analysis corresponded well with the formulation as tetramethyl tetraethylporphyrin, the possibilities for identification of the natural product through this derivative were canvassed. Using the Küster porphyrin formulation, four etioporphyrins can be written.[51]
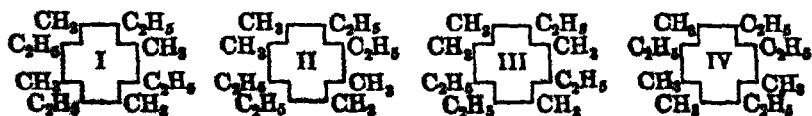
Fig. 31 *

All four of these porphyrins were synthesized and the natural product was compared with them. Unfortunately, however, no method for identification was found which was capable of proving identity unequivocally,[52] since all of them melted with decomposition at a high temper-

[50] Willstätter and Stoll, "Untersuchungen über Chlorophyll," J. Springer, Berlin (1913), p. 411. See also Willstätter and M. Fischer, Ann., 400, 182 (1913).

[51] Fischer and Stangler, Ann., 450, 62–3 (1927).

* We shall adopt Fischer's shorthand system for representing the porphyrin nucleus by omitting all the bridges and rings and representing each pyrrole ring by only a bracket for its β-positions.

[52] Fischer and Stangler, Ann., 450, 60 (1927).

ature. It was necessary to abandon this short cut, then, and to choose
a substance for the comparison which could be identified by its melting
point, even though the number of possible isomers was materially
larger. The substance chosen was mesoporphyrin, prepared as described
on p. 1262. This substance is a tetramethyl, diethyl, dipropionic acid
porphyrin, and its dimethyl ester melts low enough for identification
purposes.

Fifteen mesoporphyrins [51] are possible. Fischer set out to synthesize
as many of these as necessary to duplicate the natural product, starting
with the methenes which were most readily available. In this venture
he was much more successful, arriving at the natural product very early
in the series. This was shown to be number IX in his table of the pos-
sible isomers of mesoporphyrin and was derived from etioporphyrin III.
It was synthesized by the following reaction: [53]
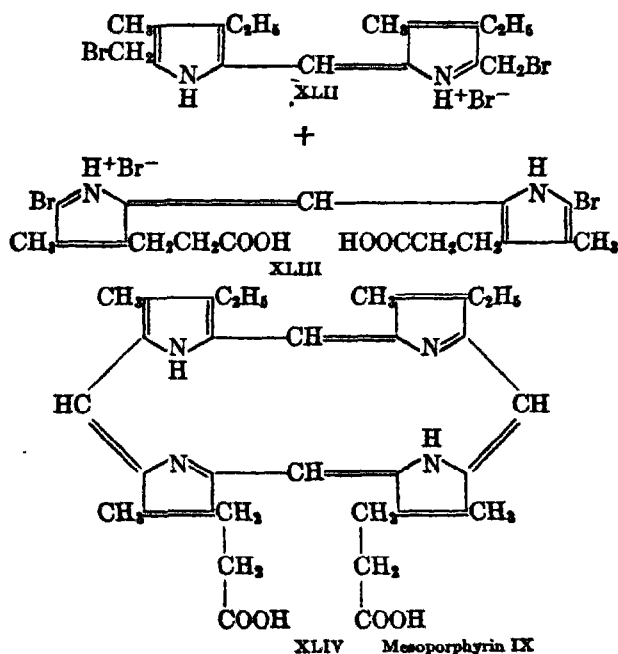
FIG. 32

The yield in this case was more than 30 per cent of the theoretical.

DEVELOPMENT OF THE HEMIN SYNTHESIS

At the time when the synthesis of mesoporphyrin was completed, it
was known that hemin contained two unsaturated side chains which
were saturated by the conversion to mesoporphyrin. Fischer believed

[54] Fischer and Stangler, Ann., 459, 72 (1927).

that one of the side chains was acetylenic and the other ethylenic.[54] However, no method existed for synthesizing porphyrins with unsaturated side chains. Fischer determined to explore the possibilities of synthesizing such porphyrins by introducing acetyl groups into free β-positions, reducing to α-hydroxyethyl groups, and dehydrating. Such a dehydration had been accomplished before in a resynthesis of hemin from hematoporphyrin,[55] which contains two molecules of water more than hemin.

Earlier it had been shown that bacteria could remove the unsaturated side chains from hemin with the formation of deuteroporphyrin.[56] Schumm then prepared deuteroporphyrin by the pyrolysis of hemin in resorcinol.[57] A synthesis was soon devised to establish the structure of this material. Because of its importance, we shall trace the course of this synthesis from the original condensations.

The starting point was ethyl methyl ketone:[58]

$$CH_3COCH_2CH_3 + RONO + HCl \rightarrow CH_3COCCCH_3 + SnCl_2 \rightarrow CH_3COCHCH_3$$
$$\underset{NOH}{\|} \qquad\qquad \underset{NH_3{}^+Cl^-}{|}$$
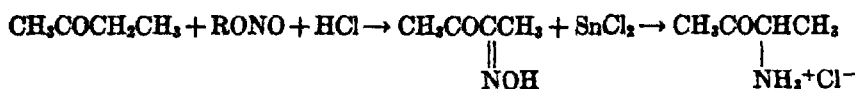
Fig. 33

α-Amino ketones of this type are stable only in the form of their hydrochlorides. When neutralized they condense with themselves or with other substances which may be present. In this instance the amino ketone was condensed with ethyl oxalacetate, XLV, by the slow addition of alkali, which also accomplished hydrolysis of the α-ester group to XLVI.[59]
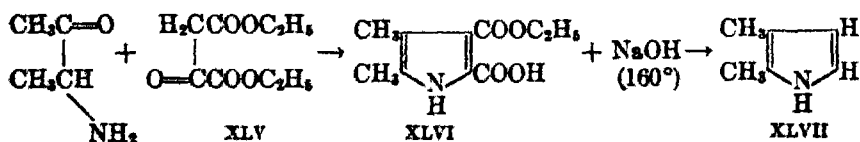
Fig. 34

A second quarter of the deuteroporphyrin was prepared from 2,4-dimethylpyrrole:[60]
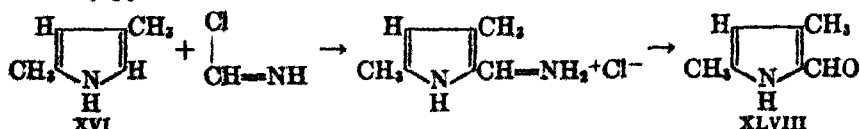
Fig. 35

[54] Fischer and Zeile, Ann., 468, 100 (1929); see also Willstätter and Stoll, "Untersuchungen über Chlorophyll," J. Springer, Berlin (1913), pp. 36-42.
[55] Fischer and Lindner, Z. physiol. Chem., 142, 141 (1925).
[56] Fischer and Lindner, ibid., 161, 17 (1926).
[57] Schumm, ibid., 176, 122 (1928); ibid., 178, 1 (1928).
[58] Fischer, Beller, and Stern, Ber., 61, 1077 (1928).
[59] Piloty and Wilke, Ber., 45, 2586 (1912); Fischer and Kutscher, Ann., 481, 199 (1930)
[60] Fischer and Zerweck, Ber., 55, 1949 (1922).

These substances were then condensed to yield a methene, XLIX, half of the deuteroporphyrin molecule.[61]
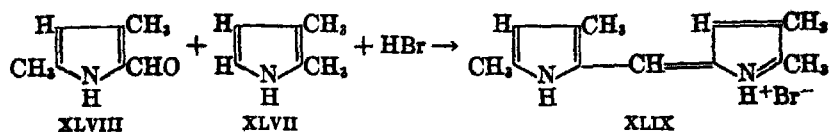
Fig. 36

The synthesis of the other half of the molecule was a longer process, starting from compound XIV, prepared above. This material hydrolyzed preferentially in the β-position when treated with concentrated sulfuric acid: [62]
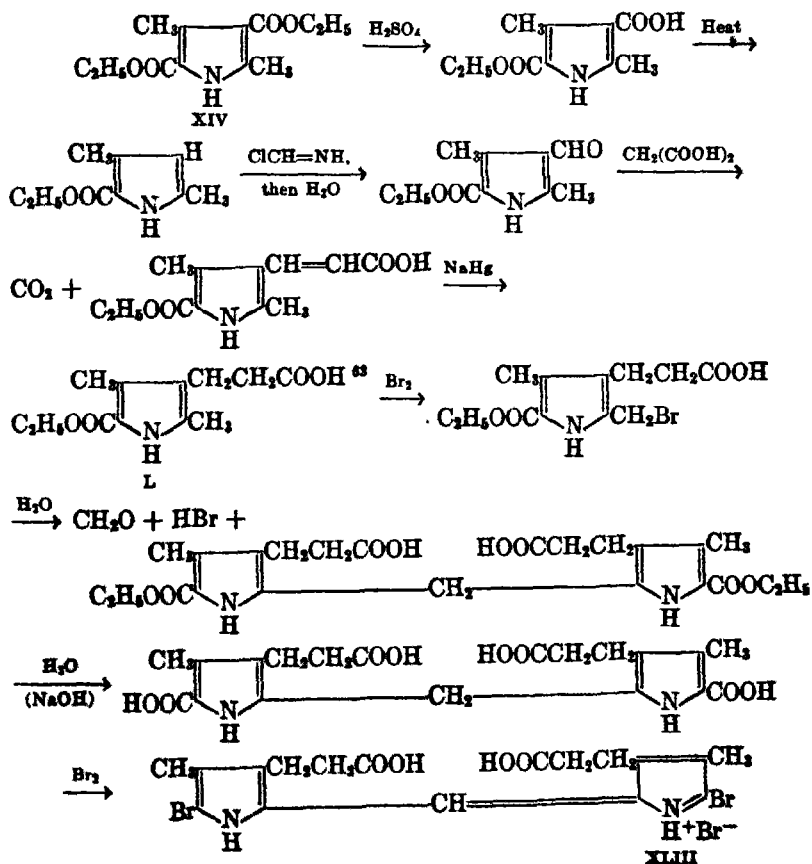
Fig. 37

[61] Fischer and Kirstahler, Ann., 466, 178 (1928).
[62] Fischer and Walach, Ber., 58, 2620 (1925).
[63] Fischer and Andersag, Ann., 450, 216 (1926).

These two methenes, XLIX and XLIII, were then combined in an acid melt to give deuteroporphyrin.[61]
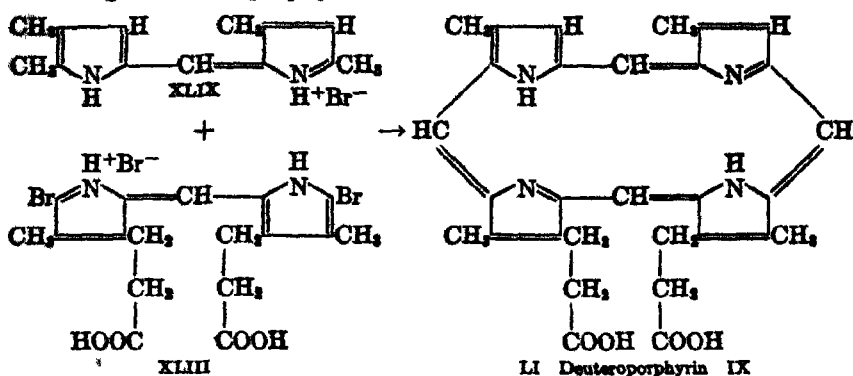
FIG. 38

The dimethyl ester of the synthetic product gave no melting-point depression with that from the natural product, and the synthesis thus demonstrated again the order of the groups in the side chains.

The introduction of two acetyl groups into the deuteroporphyrin molecule was soon achieved. In line with other observations, it was found that the Friedel-Crafts reaction proceeded more smoothly on the iron complex than on the free porphyrin. For this reason deuterohemin IX (Fig. 39, LII) was used for the synthesis:[64]
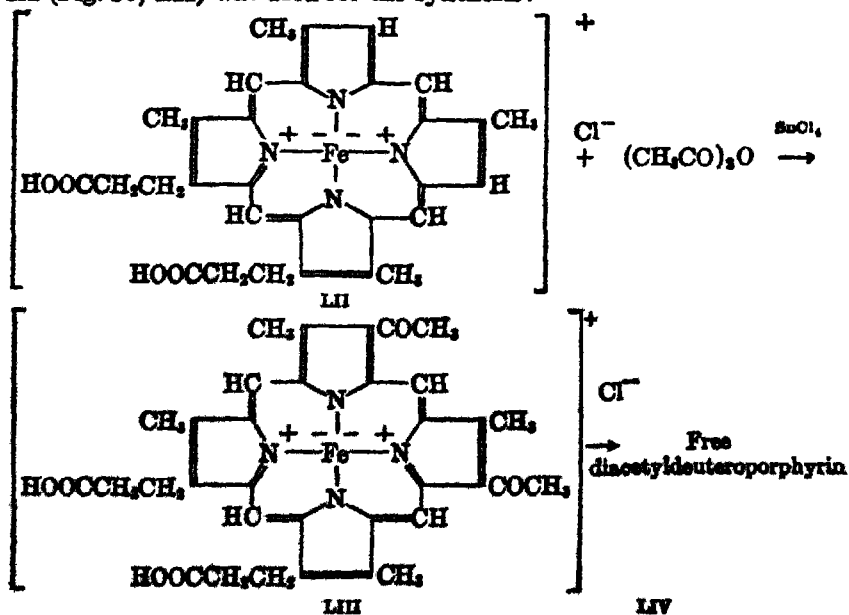
FIG. 39

64 Fischer and Zeile, Ann., 468, 98 (1929).

The reduction of this porphyrin turned out to be a matter of considerable difficulty and was solved only by recourse to a method which had lain dormant in the literature for nearly a hundred years. Dumas and Stas [65] had discovered that ethanol and potassium hydroxide react to form hydrogen and potassium acetate according to the following equation:

$$C_2H_5OH + KOH \rightarrow CH_3COOK + 2H_2$$
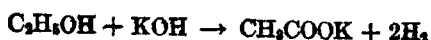
Fischer found that, in the presence of a reducible substance, the reaction led to reduction of the organic residue:

LIV

LV
Hematoporphyrin

Fig. 40
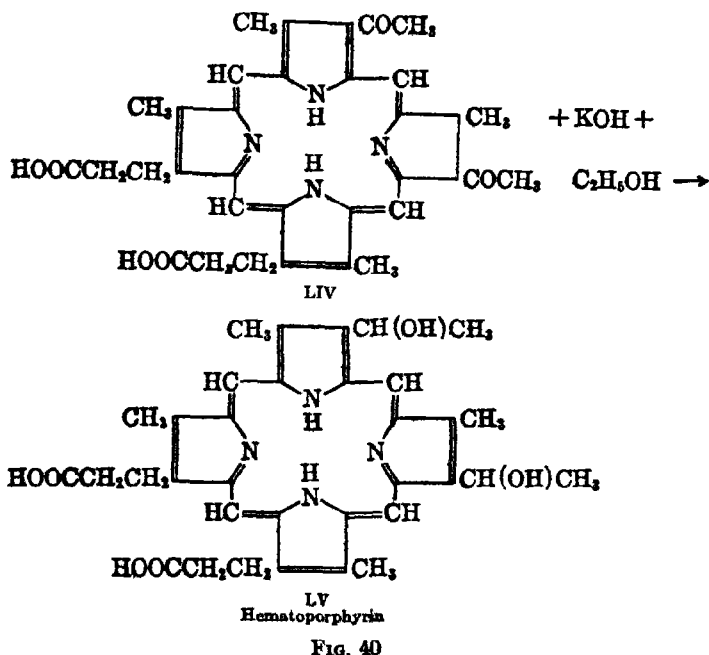
The porphyrin obtained from this reduction proved to be identical in all respects with natural hematoporphyrin, a result which was not expected at the time. Since the material was hematoporphyrin, the theory that hemin had one acetylenic link had to be discarded, for the process used in the synthesis could hardly be interpreted as leading to the formation of the enolic side chain required by an acetylenic derivative. Accordingly it became necessary to formulate hemin with two ethylenic side chains in the 2- and 4-positions. The remainder of the hemin synthesis had been completed previously, starting with naturally obtained degradation products. From hematoporphyrin, protopor-

[65] Dumas and Stas, Ann., 35, 132 (1840); C. Hell, Ann., 223, 269 (1884).

phyrin was obtained by dehydration, and, from this, hemin was prepared by introduction of iron:
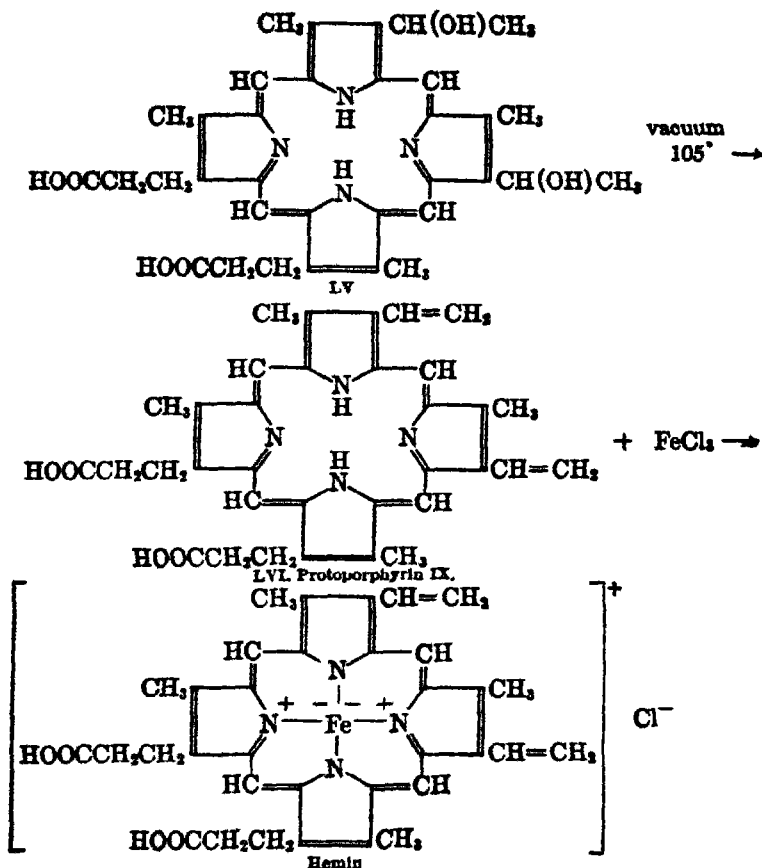
FIG. 41

FURTHER CONSIDERATION OF THE STRUCTURE OF HEMIN

It will readily be appreciated, after perusal of the foregoing section, that the configurational studies on hemin rest entirely upon synthetic methods. During the past few years it has become increasingly apparent that structural proofs based upon the aldehyde synthesis of methenes may be open to question in certain cases. Anomalous reaction products have now been obtained from a number of methene syntheses.[66]

[66] Corwin and Andrews, J. Am. Chem. Soc., 58, 1086 (1936); Corwin and Andrews, ibid., 59, 1973 (1937); Paden, Corwin, and Bailey, ibid., 62, 418 (1940).

It has been shown that the aldehyde synthesis of methenes may yield at least three different products. If pyrrole rings with different substituents in the 1-, 3-, 4-, and 5-positions are marked by distinctive numbers, these products may be represented as follows:
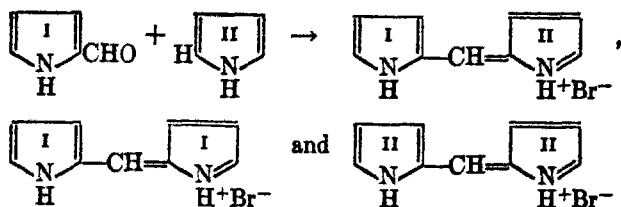
Fig. 42

Thus the normal unsymmetrical methene I–II may not appear and may be replaced by either of the symmetrical methenes, I–I or II–II. These difficulties are easily recognized if the alternative reaction products vary widely in analysis. If they are isomeric, however, the situation becomes more complicated. Reference to the synthesis of deuteroporphyrin will show that the synthesis of the tetramethyl methene, XLIX, used in this series, is just such a case. All three possible methenes would have the same analysis.

This methene synthesis has now been reinvestigated in the light of the known methene anomalies.[67] Each of the symmetrical methenes has been prepared and characterized:
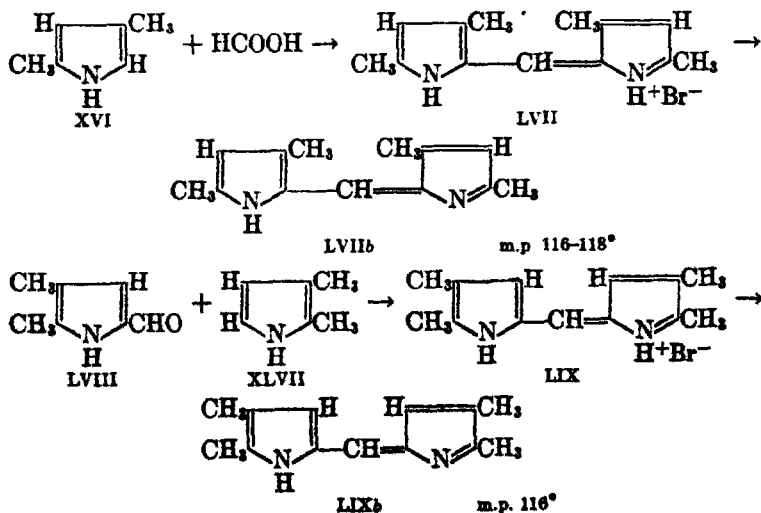
Fig. 43

[67] Corwin and Krieble, *ibid.*, **63**, 1829 (1941). See also Fischer and Endermann, *Ann.* **545**, 148 (1940).

In the latter aldehyde condensation there is no possibility of an unsym metrical product.

When the condensation used in the deuteroporphyrin synthesis, Fig 36, was repeated under anhydrous conditions, a methene was obtained which was different from either of the symmetrical methenes. It would be inferred, then, that this was the unsymmetrical methene, since i gave melting-point depressions with each of the symmetrical methenes and showed the correct analysis. Its melting point was 32° lower than that recorded by Fischer for the material used in the hemin synthesis, however. Since Fischer's melting point corresponded to the symmetrical methenes LVIIb and LIXb, it might be inferred that the wrong intermediate had appeared in the hemin synthesis. Accordingly the corresponding deuteroporphyrins were prepared by appropriate condensations. These were found to be three individuals, easily characterized. The unsymmetrical methene gave natural deuteroporphyrin. Thus it is apparent that the aldehyde synthesis did not follow an anomalous course. The structure of hemin as originally formulated is confirmed by this work, but the physical properties of the intermediate methene must be revised.

THE "FINE STRUCTURE" OF THE PORPHYRIN NUCLEUS

The question of the so-called fine structure of the porphyrin nucleus is one which has received some attention in the literature [68] but one on which decisive experiments are still lacking. Fischer early considered the possibility that porphyrins might exist in isomeric forms depending upon the positions of the hydrogens on the nitrogens of the nuclei.[69]
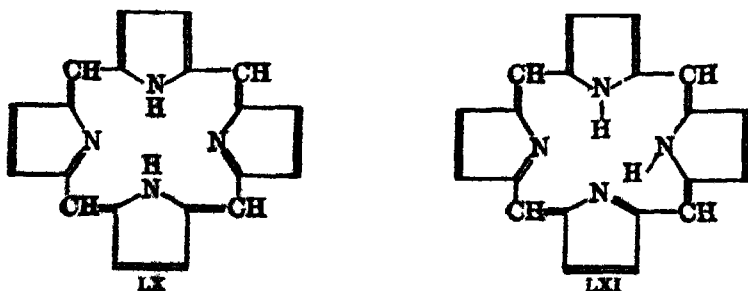
Fig. 44

While isomers of this type might be tautomeric and capable of independent existence, all differences between the two types would disappear upon conversion to a metallic complex or to an acid salt:

[68] For a summary of the literature see Fischer-Orth "Die Chemie des Pyrrols," Akad. Verlag., Leipzig (1937), Bd. II, 1 Hälfte, pp. 172, 233.

[69] Fischer and Stangler, Ann., 459, 54 (1927).
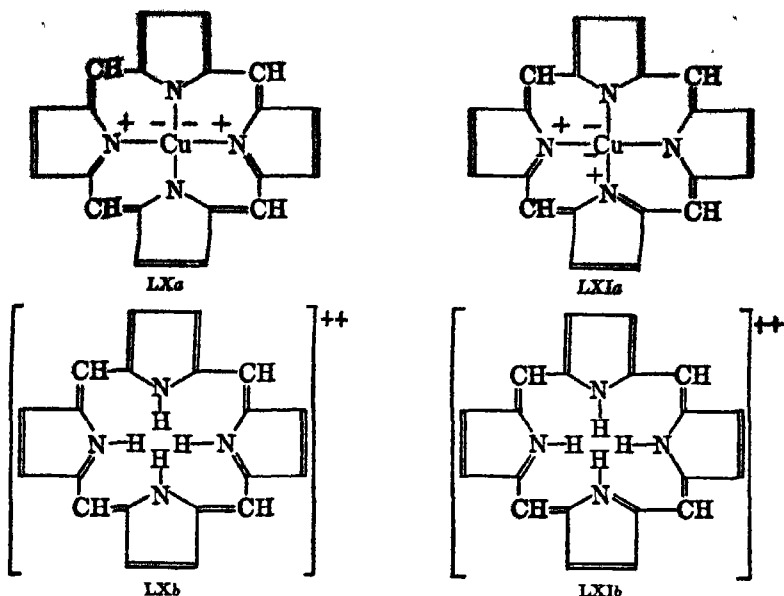
Fɪɢ. 45

Inspection of these formulations will show that they are electronic isomers since models of them require no shift of atomic nuclei for interconversion. Therefore, according to all the rules of experience, they should not be capable of differentiation, just as it has proved impossible to isolate isomeric forms of ortho-disubstituted benzene derivatives. For this reason it seems improbable that isomers which have been isolated from an acid melt or purified by acid fractionation or which give different metallic complexes can be formulated as "N-isomers" of this type.[70]

Huggins[71] suggested another possibility for isomerism which might exist in certain unsymmetrically substituted porphyrins:
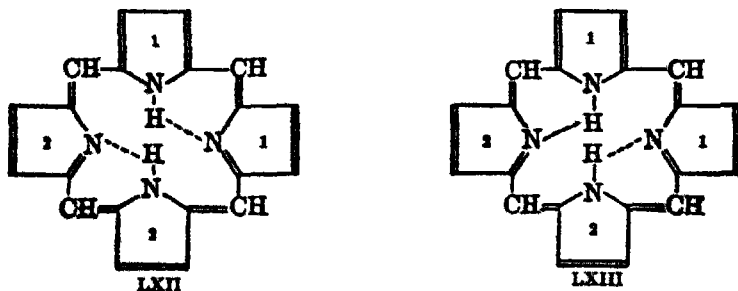
Fɪɢ. 46

[70] Conant and Bailey, J. Am. Chem. Soc., 55, 795 (1933); Corwin and Quattlebaum, ibid., 58, 1081 (1936); compare Rothemund, ibid., 61, 2912 (1939).

[71] See Corwin and Quattlebaum, ibid., 58, 1082 (1936), footnote 6.

Construction of a model of this type of compound shows that the interconversion of the isomers should require a marked shift of atomic centers, the bridged nitrogens being closer together than the unbridged ones.   This NH-bridge type of isomerism should also disappear on acidic salt or metallic complex formation, however.

In his studies of the structure of phthalocyanin, LXIV, by the x-ray method, Robertson [72] showed that pairs of adjacent nitrogen atoms were closer together than opposite ones.   This made it seem probable that the force joining these atoms was due to hydrogen bridging from N to N:

LXIV
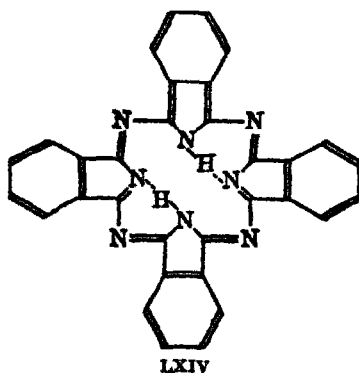
FIG. 47

This work on a closely related substance makes it appear quite probable that porphyrins will also be shown to have NHN bridges joining adjacent pyrrole nuclei.

Various attempts have been made to adduce the "positions" of double bonds in the porphyrin nucleus by physical methods such as the measurement of absorption spectra and magnetic properties.   These can hardly receive serious consideration until a much larger mass of data has been accumulated or until the theory concerning them has been refined sufficiently to give confidence as to their reliability.   In view of past experience with smaller resonating systems, it seems that any attempt to formulate fixed bonds in the large conjugated system of the porphyrin ring will remain arbitrary for some time and will then have to withstand controversial fire before it can be accepted.

It will be noted that, in the coördination complex hemin, iron is tetracovalent.   Studies of iron complexes have shown that the hexacovalent state is of much more frequent occurrence [73] and suggest that

[72] Robertson, J. Chem. Soc., 1195 (1936); Robertson and Woodward, ibid., 219 (1937).
[73] See, for instance, Ephraim, "Anorg. Chem.," Verlag Steinkopf, Dresden (1934), 5 Aufl., pp. 268-271.

a complex such as hemin should readily add substances capable of coördination. That this is true is shown by the formation of complexes with a large variety of organic bases. Surprisingly enough, these complexes are relatively unstable. The ability to coördinate seems to be the basis for the utilization of hemin in hemoglobin. Globin, a high-molecular-weight protein base, is capable of forming a very stable coördination complex with the reduced form of hemin to make hemoglobin. Furthermore, this base so modifies the nature of the iron atom that it becomes capable of combining reversibly with molecular oxygen. The reasons for these chemical peculiarities of globin are unknown at the present time, and the search for them will be one of the interesting chemical investigations in this field.

OTHER PORPHYRINS

Various porphyrins not directly related to hemin have been found in natural sources or have been prepared from natural products. The most important of these are the koproporphyrins, the uroporphyrins, and the chlorophyll porphyrins.

Koproporphyrin I, corresponding in its arrangement of side chains to etioporphyrin I (see p. 1278), with the exception that the four ethyl groups are replaced by four propionic acid groups, was first isolated from feces.[74] It has also been isolated from urine and from various organs in cases of porphyrinuria, or porphyria, in which abnormally large amounts of porphyrins are excreted.[75] Free porphyrins in the blood stream give rise to hypersensitivity to light, and victims of porphyrinuria who do not eliminate the porphyrins rapidly enough suffer severely from even moderate exposures to light. The structure of this compound has been established by synthesis.[76] In certain cases koproporphyrin III, corresponding in structure to etioporphyrin III, also appears in the excreta. Uroporphyrin I [77] contains eight carboxyl groups and can be decarboxylated to koproporphyrin I, but the positions of the extra four carboxyl groups have not yet been definitely assigned.[78]

[74] Fischer, Z. physiol. Chem., 96, 156 (1915).

[75] Fischer, Hilmer, Lindner, and Pützer, ibid., 150, 44 (1925). For a complete discussion see Vannotti, "Porphyrine und Porphyrinkrankheiten," J. Springer, Berlin (1937).

[76] Fischer and Andersag, Ann., 450, 212 (1926).

[77] Fischer, Z. physiol. Chem., 95, 43 (1915).

[78] Fischer and Zischler, ibid., 245, 123-38 (1937); Fischer and Hofmann, ibid., 246, 15-30 (1937); Fischer and Müller, ibid., 246, 31-42 (1937).

THE CONFIGURATION OF CHLOROPHYLL

By far the largest group of porphyrins numerically has been obtained from one or another of the multitudinous degradations of chlorophyll. Since the variety of the side chains on the chlorophyll porphyrins is larger than that in the hemin porphyrins, the possibilities for isomerism are correspondingly increased and the configurational problem, attacked from the synthetic point of view, becomes correspondingly more difficult. We shall consider the proof of the structure of pyrroporphyrin, a substance obtained by drastic alkaline degradation of chlorophyll derivatives.

By analysis and degradation pyrroporphyrin was shown to be a tetramethyl, diethyl, monopropionic acid porphyrin.[79] Twenty-four isomers of this compound can be written. By filling the vacant β-position with an ethyl group, however, the number of possible isomers is reduced to eight.[80] Experimentally, this synthetic operation was carried out on pyrroporphyrin through the iron complex as follows: [81]

$$\underset{\text{Pyrroporphyrin}}{\overset{\text{H}\diagdown}{\underset{\text{H}\diagup}{>}}\text{RH}} + \text{Fe Acetate} + \text{NaCl} \rightarrow \underset{\text{Pyrrohemin}}{(\text{Fe}>\text{RH})^{+}\text{Cl}^{-}} + (CH_3CO)_2O \xrightarrow{\text{SnCl}_4}$$

$$\underset{\text{Acetyl pyrrohemin}}{(\text{Fe}>\text{RCOCH}_3)^{+}\text{Cl}^{-}} + \text{HCl} \rightarrow \underset{\text{Acetyl pyrro-porphyrin}}{\overset{\text{H}\diagdown}{\underset{\text{H}\diagup}{>}}\text{RCOCH}_3} + \text{KOH} + C_2H_5OH \rightarrow$$

$$\underset{\substack{\text{Hydroxyethyl}\\\text{pyrroporphyrin}}}{\overset{\text{H}\diagdown}{\underset{\text{H}\diagup}{>}}\text{RCH(OH)CH}_3} + \text{HI} \rightarrow \underset{\substack{\text{Ethyl pyrro-}\\\text{porphyrin}\\\text{LXV}}}{\overset{\text{H}\diagdown}{\underset{\text{H}\diagup}{>}}\text{RCH}_2\text{CH}_3}$$
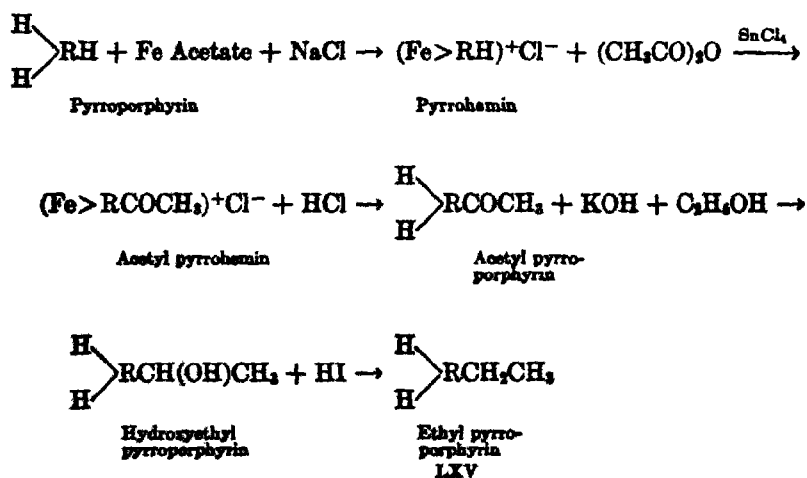
Fig. 48

This ethylpyrroporphyrin is, then, a tetramethyl triethyl monopropionic acid porphyrin. All eight of these substances were synthesized, and the last one prepared proved to be the desired ethylpyrroporphyrin: [81]

[79] Willstätter and Fritzsche, Ann., 371, 94 (1909); Fischer, Berg, and Schormüller, Ann., 480, 109 (1930).

[80] Fischer, Grosselfinger, and Stangler, Ann., 461, 222 (1928).

[81] Fischer, Weichmann, and Zeile, Ann., 475, 253–257 (1929).
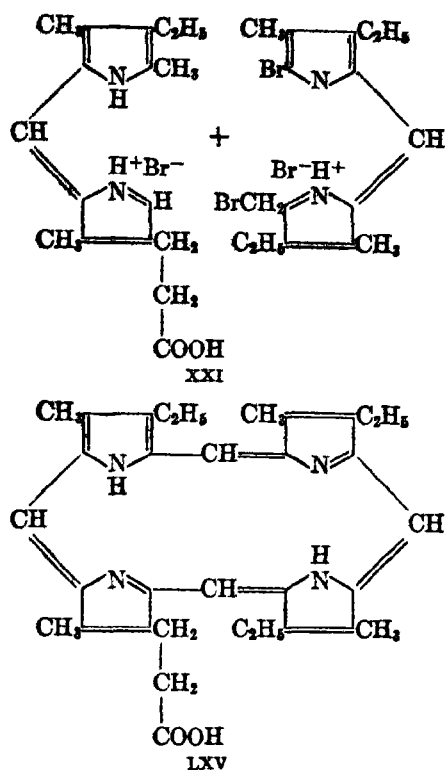
FIG. 49

This synthesis led Fischer to the surprising conclusion that the red blood pigment and the green leaf pigment have essentially the same arrangement of side chains.

With the general arrangement of the side chains established, the possibilities for the structure of pyrroporphyrin were reduced to three: the free position could be any one of those occupied by an ethyl group, 2, 4, or 6. These compounds were synthesized, and it was determined that the 6-position was the one actually open.[46a] It had been determined previously that rhodoporphyrin differed from pyrroporphyrin only in the presence of a carboxyl group which replaced the free position of pyrroporphyrin.[82] Thus the way was immediately open to the synthesis of rhodoporphyrin,[46b] which was accomplished at almost the same time by the methods sketched earlier in this paper. These syntheses solved the problem of the arrangement of the groups in the

[82] See Willstätter and Stoll, "Untersuchungen über Chlorophyll," J. Springer, Berlin (1913), p. 347.

chlorophyll porphyrins and laid the foundation for the further work on the minutiae of chlorophyll structure which will be summarized in the succeeding chapter.

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CHAPTER 17

CHLOROPHYLL

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CONTENTS

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INTRODUCTION

The name "chlorophyll" was first given by Pelletier and Caventou (1817) to the green coloring material present in the chloroplasts of green plants, especially in the leaves. In 1818 the English physicist Stokes [1] showed spectroscopically that the chloroplast pigment was a mixture, and Sorby [2] separated it into four pigments, two yellow and two green, by partition between immiscible solvents. This work was ignored until Willstätter (1906) began his investigations. Meanwhile many attempts were made to isolate the chloroplast pigments; the isolation of the yellow pigments was achieved by several investigators, but the use of too drastic methods gave very impure chlorophyll extracts, so that many workers reported a different chlorophyll for each plant species.

From 1906 to 1914 Willstätter [3] and his collaborators not only succeeded in preparing relatively pure chlorophyll for the first time, but also by their chemical investigations laid the foundation of our knowledge of the structure of the green pigments. Willstätter showed that two green pigments, chlorophylls a and b, were present in all plant leaves, and that they could be separated by the preferential solubility of chlorophyll a in petroleum ether and of chlorophyll b in 90 per cent methyl alcohol. On the basis of the earlier discovery of Tswett [4] of the use of chromatographic adsorption as a means of separating pigments in solution, Winterstein and Stein [5] separated the two chlorophylls by adsorption on powdered sugar. Willstätter found that the ratio of chlorophyll a to chlorophyll b was remarkably constant in the higher plants, being about three to one. The ratio is much higher for some algae, and at least three algae appear entirely devoid of chlorophyll b. [6]

Chlorophyll and its derivatives are very sparingly soluble in most of the common organic solvents and are difficult to purify. The chief means of identifying individual compounds is by their unique absorption spectra. Willstätter introduced a general method for the separation and purification of chlorophyll derivatives, by extracting them from ether solution with hydrochloric acid of different concentrations. This method of acid fractionation depends on the large differences in the dis-

[1] Stokes, *Ann. chim. phys.*, Ser. 2, **9**, 194 (1818); also *Proc. Roy. Soc.* (*London*), **13**, 144 (1864).

[2] Sorby, *Proc. Roy. Soc.* (*London*), **21**, 442 (1873).

[3] Willstätter and Stoll, "Untersuchungen über Chlorophyll," Berlin (1913); translated by Schertz and Merz, Science Publishing Co. (1928). The original papers are in the *Annalen* from 1906 to 1914.

[4] Tswett, *Ber.*, **41**, 1352 (1908).

[5] Winterstein and Stein, "Handbuch der Pflanzen Analyse," IV, II, 1403 (1932); also Z. *physiol. Chem.*, **220**, 263 (1933); **230**, 139 (1934).

[6] Fischer and Breitner, *Ann.*, **522**, 151 (1936).

tribution ratios of these substances between ether and dilute acid, brought about by small differences in basicity and in solubility. Chlorophyll derivatives are therefore all characterized by a *hydrochloric acid number*, defined as that percentage concentration of hydrochloric acid which extracts two-thirds of the substance from an equal volume of an ether solution.

Willstätter determined the correct empirical formula for chlorophyll *a* as $C_{55}H_{72}N_4O_5Mg$ (later substantiated by Fischer [7] and by Stoll [8]), and found that chlorophyll *b* contained one oxygen atom more and two hydrogen atoms less than *a*. He proved for the first time the presence of magnesium in the chlorophyll molecule, and showed that hydrolysis split off two alcohols, methyl alcohol and phytol, $C_{20}H_{39}OH$ (p. 1297).

NUCLEAR STRUCTURE OF CHLOROPHYLL

The Chlorophyll Porphyrins. The earliest obtained derivatives of chlorophyll were the pyrroles and the porphyrins. Nencki [9] and later Willstätter [10] reduced chlorophyll to substituted pyrroles, identified by Willstätter as hemopyrrole (p. 1263, formula V), cryptopyrrole (VI), and phyllopyrrole (VIII). Küster [11] obtained pyrroles by the oxidative degradation of chlorophyll, and isolated hematinic acid (p. 1263, formula IV) and ethylmethylmaleimide (I). Hemoglobin, on reduction and oxidation, respectively, gives the same series of pyrroles, as shown in the preceding chapter.

Again chlorophyll, like hemin, on drastic alkaline degradation gives porphyrins (p. 1290). The chlorophyll porphyrins were originally obtained by Hoppe-Seyler [12] and also by Schunk.[13] Indeed, this formation of porphyrins was the first indication of a similarity in chemical structure between the blood pigment and chlorophyll. The structure of the porphyrins as interpreted by the Küster formula has already been fully discussed.

In the series of porphyrins obtainable from chlorophyll, Willstätter characterized the dicarboxylic acid rhodoporphyrin (II), the monocarboxylic acids pyrroporphyrin (III), and phylloporphyrin (IV), and the oxygen-free compound pyrroetioporphyrin (I). Rhodoporphyrin can be converted into pyrroporphyrin by pyrolysis, with the loss of a molecu-

[7] Fischer and Siebel, *Ann.*, **499**, 84 (1932).

[8] Stoll and Wiedemann, *Naturwissenschaften*, **20**, 628 (1932).

[9] Nencki and Zaleski, *Ber.*, **34**, 997 (1901).

[10] Willstätter, *Ann.*, **373**, 227 (1910); **385**, 188 (1911).

[11] Küster, *Z. physiol. Chem.*, **82**, 463 (1912).

[12] Hoppe-Seyler, *ibid.*, **3**, 339 (1879); **4**, 193 (1880).

[13] Schunk, *Proc. Roy. Soc.* (*London*), **50**, 302 (1891); **57**, 314 (1895); *Ann.*, **284**, 81 (1894)

lar proportion of carbon dioxide. Pyrroporphyrin, in turn, can be decomposed with loss of carbon dioxide to give the oxygen-free, alkylated porphyrin (pyrroetioporphyrin [I]).
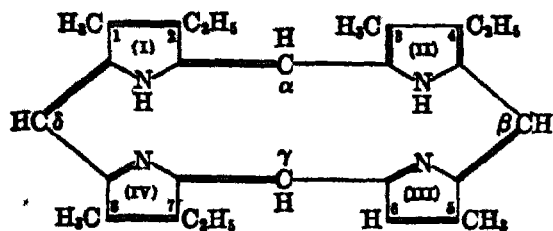
I. Pyrroetioporphyrin.

FIG. 1

Mesoetioporphyrin ("etioporphyrin III"), the simplest blood porphyrin, differs from this in containing an ethyl group in position 6. That this position was unsubstituted in the chlorophyll porphyrins, phyllo-, pyrro-, and pyrroetioporphyrins, was shown by bromination followed by oxidation. Bromine replaces the hydrogen in the unsubstituted
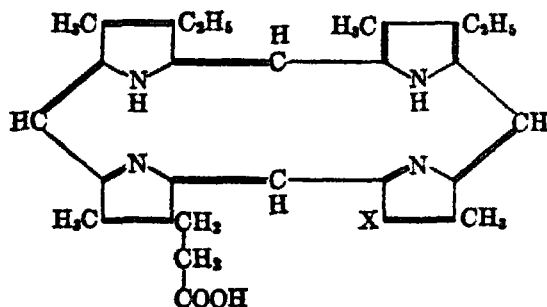
II. Rhodoporphyrin X = COOH
III. Pyrroporphyrin X = H
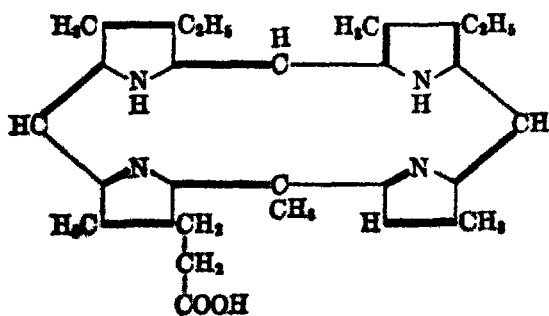
IV. Phylloporphyrin.

FIG. 2

position, and on oxidation, porphyrin ring fission occurs and bromo-citraconimide is obtained.[14]

Hans Fischer synthesized many series of isomeric porphyrins; among these were porphyrins [15, 16] identical with rhodoporphyrin (II), pyrroporphyrin (III), and phylloporphyrin (IV) from chlorophyll. Syntheses of rhodoporphyrin and of 6-ethylpyrroporphyrin have already been outlined (pp. 1275, 1290).

Natural phylloporphyrin, which contains a methyl group on the γ-bridge carbon atom (see Fig. 1 for system of numbering), can be converted into pyrroporphyrin by the action of sodium ethoxide.

Rhodoporphyrin, as shown above, possesses besides a propionic acid group in position 7 a carboxyl group in the 6-position, which is occupied by the unsubstituted hydrogen atom in pyrro- and phylloporphyrins. In systematic nomenclature, rhodoporphyrin is 1,3,5,8-tetramethyl-2,4-diethyl-6-carboxyporphyrin-7-propionic acid.

CHLOROPHYLL a

Willstätter's Investigations. Willstätter obtained a number of derivatives of chlorophyll by the action of acid and alkali. For instance, treatment with oxalic acid removes the magnesium and yields a waxy substance called pheophytin (V). This has no acid properties, and therefore the magnesium must be attached to nitrogen, and not to an acidic group as a salt. Hydrolysis of pheophytin with strong acid removes phytol, yielding pheophorbides a and b, both monomethyl esters of dicarboxylic acids (VII). These can also be obtained directly from chlorophyll by the action of strong mineral acid. Esterification gives the dimethyl esters, methyl pheophorbides a and b. Since the pheophorbides are more readily separated into the a and b components by acid fractionation than are the magnesium-containing chlorophylls or pheophytin, the separation is usually carried out at this stage.

If sections of the green leaves of certain plants (e.g., *Heracleum spondylium*) are soaked in alcohol, large crystals are developed in the chloroplasts; the substance produced was called "crystallized chlorophyll." Willstätter showed that these plants contain an enzyme, chlorophyllase, which hydrolyzes the phytyl group and replaces it by the alcohol present. The products are now called ethylchlorophyllide (IX), if ethyl alcohol is used, methylchlorophyllide from methyl alcohol, and so forth.

[14] Fischer and Treibs, *Ann.*, **466**, 188 (1928).
[15] Fischer, Berg, and Schormüller, *Ann.*, **480**, 109 (1930).
[16] Fischer and Helberger, *Ann.*, **480**, 235 (1930).

More research has been done on the structure of the *a* series than on that of the *b* series for two reasons: (1) pheophytin *a* and especially methyl pheophorbide *a* are more easily obtained pure in the separation of the *a* and *b* components; (2) the *a* series gives cleaner reactions with fewer decomposition by-products, for reasons obvious later. Chlorophyll *a* will therefore be considered first. Saponification of pheophorbide *a* or of its ester with hot alkali yields phytochlorin *e* (abbreviated to chlorin *e* or e_6 since it contains six oxygen atoms), which gives with diazomethane a trimethyl ester.[17] These reactions of chlorophyll *a* are summarized as follows:

VI. Chlorophyll *a*

$C_{32}H_{30}N_4OMg(COOCH_3)$
$(COOC_{20}H_{39})$

Oxalic acid

Strong acid

Chlorophyllase in ethyl alcohol

V. Pheophytin *a* ——Strong acid——→ VII. Pheophorbide *a* ←——Acid—— IX. Ethyl chlorophyllide *a*

$C_{32}H_{30}N_4O(COOCH_3)$
$(COOC_{20}H_{39})$

$C_{32}H_{32}N_4O(COOCH_3)$
$(COOH)$

$C_{32}H_{30}N_4OMg(COOCH_3)$
$(COOC_2H_5)$

Hot alkali

Hot alkali

Alkali, then acid

VIII. Chlorin *e*

$C_{31}H_{32}N_4(COOH)_3$
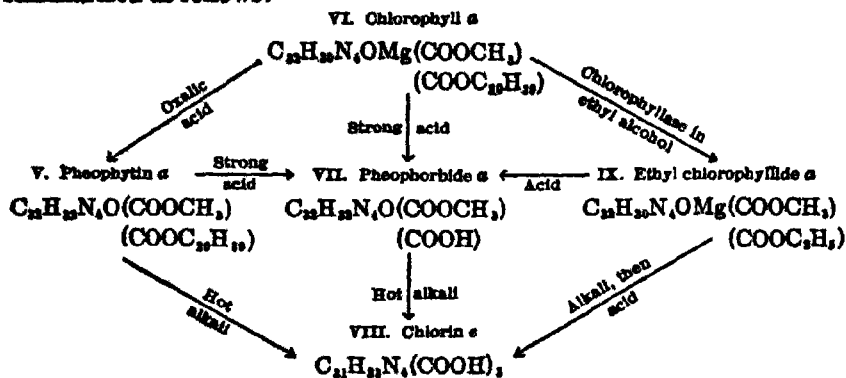
FIG. 3

After the brilliant syntheses of the chlorophyll porphyrins by Hans Fischer, the relationship of the structure of chlorophyll to the porphyrin nucleus and the nature of the labile groups in chlorophyll required elucidation. The problem was taken up almost simultaneously by Fischer and by J. B. Conant. A. Stoll and L. Marchlewski have also made contributions.

The Phytyl Group. Conant[18, 19] first correctly placed the phytyl group in chlorophyll on the propionic acid side chain (Fig. 2). Pheophorbide *a* contains a methoxyl group derived unchanged from chlorophyll *a* (Fig. 3) and a free acid residue resulting from the loss of phytol through hydrolysis. On pyrolysis, pheophorbide *a* is converted into pyropheophorbide *a* (XIII), which has lost a carbomethoxyl group (—COOCH₃) but still retains a carboxylic acid group. This last group must be the propionic acid residue; otherwise it would be eliminated in the pyrolysis. Hence the propionic acid group which survives pyrolysis must be esterified with phytol in the chlorophyll molecule.

[17] Treibs and Wiedemann, *Ann.*, 471, 146 (1929).
[18] Conant and Hyde, *J. Am. Chem. Soc.*, 51, 3668 (1929).
[19] Conant, Dietz, Bailey, and Kamerling, *ibid.*, 53, 2382 (1931).

The Hydrogen Iodide Reaction. Fischer found a mild reagent for the degradation of chlorophyll in glacial acetic acid solutions of hydrogen iodide at 50° C. By such treatment, many chlorophyll derivatives are reduced to colorless leuco compounds, which reoxidize in air to porphyrins;[20, 21, 22, 23] these, however, unlike the chlorophyll porphyrins previously described, retain the full carbon skeleton of the original compound. In this manner the pheophorbides give rise to the pheoporphyrins, while chlorin *e* gives the chloroporphyrins. Thus pheophorbide *a* with hydrogen iodide yields pheoporphyrin a_5, $C_{35}H_{36}O_5N_4$ [22] (Fig. 5); this isomer of pheophorbide *a* (see p. 1306) contains, like the parent compound, a carbomethoxyl group and a carboxyl group. Further treatment with hydrogen iodide, or the action of hydrogen bromide in acetic acid, eliminates the carbomethoxyl group, giving phylloerythrin, $C_{33}H_{34}O_3N_4$, which is spectroscopically a porphyrin. Phylloerythrin may be obtained directly from pheophorbide *a*, pheophytin, and the chlorophyllides, by refluxing them with 20 per cent hydrochloric acid. Now phylloerythrin was already known as a biological decomposition product of chlorophyll; Loebisch and Fischler,[24] finding it in ox bile, called it bilipurpurin, while Marchlewski[25] isolated it from the feces of herbivora. These biological sources of phylloerythrin convinced Fischer that the hydrogen iodide treatment was mild, and that chlorophyll, the phorbides, and phylloerythrin were closely related in chemical structure.

Phylloerythrin contains a reactive carbonyl group which forms an oxime, and which can be reduced by the Wolff-Kishner method (hydrazine and sodium ethoxide in a sealed tube) to form desoxophylloerythrin (X), $C_{33}H_{36}O_2N_4$.[23] This last may be obtained directly from pheophytin *a*, by heating with formic acid to 160° C., or from pheophorbide *a*, by the action of hydrogen bromide in acetic acid at 180° C.[26] The two oxygen atoms in this porphyrin belong to a propionic acid group. Drastic treatment with sodium ethoxide in the presence of air converts desoxophylloerythrin into phyllo-, pyrro-, and rhodoporphyrins (Fig. 2). Fischer had synthesized all the possible isomeric tetramethyl-triethylporphyrinpropionic acids,[27] from which desoxophylloerythrin differs in formula by only two hydrogen atoms, but found that they differed widely in properties. Fischer concluded that the new compound

[20] Fischer, Merkx, and Plötz, *Ann.*, **478**, 283 (1930).

[21] Fischer and Bäumler, *Ann.*, **480**, 197 (1930).

[22] Fischer and Süs, *Ann.*, **482**, 225 (1930).

[23] Fischer, Moldenhauer, and Süs, *Ann.*, **485**, 1 (1931).

[24] Loebisch and Fischler, *Monatsh.*, **24**, 335 (1903).

[25] Marchlewski, *Z. physiol. Chem.*, **43**, 464 (1904); **45**, 466 (1905).

[26] Fischer, Moldenhauer, and Süs, *Ann.*, **486**, 107 (1931).

[27] Fischer, Grosselfinger, and Stangler, *Ann.*, **461**, 221 (1928); Fischer, Weichmann, and Zeile, *Ann.*, **475**, 241 (1929).

was a tetramethyldiethylporphyrinpropionic acid with the additional C_2H_2 forming a five-membered ring between the γ- and 6-positions, the so-called carbocyclic or isocyclic ring. Such a compound would give phyllo- and rhodoporphyrins on degradation. Fischer proved this structure (X) by synthesis.[28] Desoxophylloerythrin can be oxidized
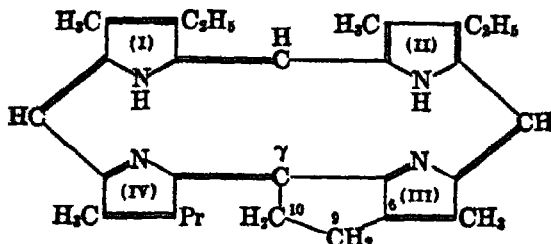
X. Desoxophylloerythrin (Pr = —CH$_2$CH$_2$COOH).

FIG. 4

with potassium dichromate and sulfuric acid to regenerate phylloerythrin. Phylloerythrin contains a carbonyl group in place of one of the CH_2 groups, and that this is in position 9 and not 10 follows from its alkaline decomposition to rhodoporphyrin, containing a carboxyl group in position 6 (II).

The structure of pheoporphyrin a_5[28] (XI) is established by its relationship to phylloerythrin and the chloroporphyrins. Pheoporphyrin a_5 contains a carbomethoxyl group and a carbonyl group. On hydrolysis it yields chloroporphyrin e_6, so called because its monomethyl ester is obtainable by the action of hydrogen iodide on chlorin e_6 trimethyl ester. Chloroporphyrin e_6 is rhodoporphyrin-γ-acetic acid; formic acid converts it to γ-methylrhodoporphyrin (chloroporphyrin e_4), and this in turn can be decarboxylated to phylloporphyrin (IV). Pheoporphyrin a_5 therefore contains the carbocyclic ring of phylloerythrin with a carbomethoxyl group in position 10. Figure 5 summarizes these relationships. Partial formulas indicate the structure of the compounds between nucleus (III) and the γ-methene bridge. In the erythrins and the porphyrins, but not in the phorbides and chlorins, the remainder of the formulas is identical with that of desoxophylloerythrin (X). Other proofs of the position of the carbonyl group in position 9 in pheoporphyrin a_5 and in phylloerythrin have been found through partial syntheses of these substances. For instance, phylloerythrin was obtained by a condensation between positions 6 and γ in chloroporphyrin e_4 (γ-methylrhodoporphyrin) under the influence of sodium ethylate.[29]

[28] Fischer and Riedmair, Ann., 490, 91 (1931).
[29] Fischer, Müller, and Leschhorn, Ann., 523, 164 (1936).
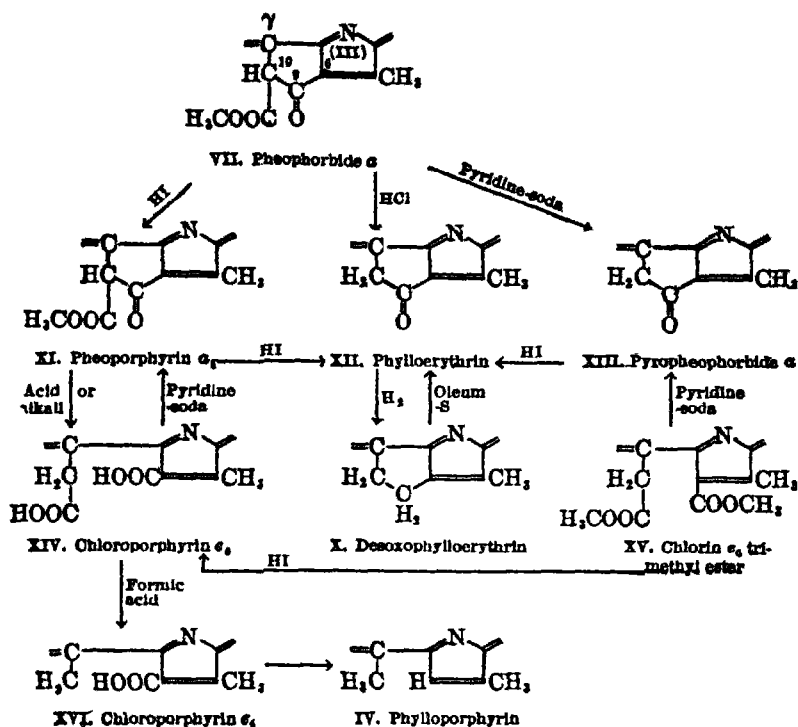
FIG. 5. The hydrogen iodide reaction.[30]

The Carbocyclic Ring. Pyrolysis [31] of pheophorbide a in pyridine and sodium carbonate eliminates the carbomethoxyl group with formation of pyropheophorbide a (XIII). This contains a carbonyl group in a carbocyclic ring, and is spectroscopically a phorbide, but is isomeric with phylloerythrin, into which it is converted by treatment with hydrogen iodide. Phylloerythrin, pyropheophorbide a, and its dihydro derivative, mesopyropheophorbide a, occur together as biological degradation products of chlorophyll in sheep feces.[32] Alkaline hydrolysis of pheophorbide a gives chlorin e_6 (Fig. 3), which contains no carbocyclic ring. Its trimethyl ester (XV) heated with pyridine and sodium carbonate forms that ring again, giving pyropheophorbide a.[33] A similar synthesis

[30] For a more complete discussion, consult the two summaries by Fischer, Ann., 502, 175 (1933); Pedler Lecture, J. Chem. Soc., 245 (1934).

[31] Fischer, Filser, Hagert, and Moldenhauer, Ann., 490, 1 (1931).

[32] Fischer and Hendschel, Z. physiol. Chem., 198, 33 (1931); 222, 250 (1933); Fischer and Stadler, ibid., 239, 167 (1936).

[33] Fischer and Siebel, Ann., 494, 73 (1932).

of the carbocyclic ring occurs in the pyrolysis of chloroporphyrin e_6 to pheoporphyrin a_5.

The carbocyclic rings of pyropheophorbide a and of phylloerythrin are the same; those of pheophorbide a and pheoporphyrin a_5 are also identical. Position 10, both in certain of the chlorophyll porphyrins and in pheophorbide a, can be oxidized by iodine.[34] For example, pheoporphyrin a_5 with iodine in alcohol containing sodium acetate is converted into the acetyl derivative of 10-hydroxypheoporphyrin a_5 (XVIII) (in the older literature "neopheoporphyrin a_6"), while the substitution of sodium carbonate for sodium acetate leads to 10-ethoxy-pheoporphyrin a_5 (XIX) (or "pheoporphyrin a_6").[35] A series of homologous ethers has been prepared by the use of the corresponding series of alcohols in this oxidation.[36] Pheophorbide a with iodine in glacial acetic acid[36] gives the acetyl derivative of 10-hydroxypheophorbide a.
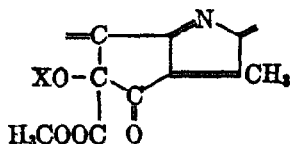
XVII. 10-Hydroxypheophorbide a or XVIII. 10-Hydroxypheoporphyrin a_5. X = H
XIX. 10-Ethoxypheoporphyrin a_5. X = C_2H_5.

FIG. 6

The chlorophyllides, methylpheophorbide, and pheophorbide at ordinary temperatures in an atmosphere of nitrogen form oximes,[35] which analyze as substitution, not addition, products, and hence show the presence of a carbonyl group. The original compounds can be regenerated from the oximes, and the oximes are converted by hydrogen iodide into the oxime of pheoporphyrin a_5. The enolic modification must also be possible, since methylpheophorbide and pheophorbide form benzoyl substitution products, as was shown by Stoll.[37] The β-ketonic acid grouping (RCOCHR'COOCH₃) of the carbocyclic ring admits of these modifications and explains the reaction of chlorophyll and the pheophorbides to acid and to hot alkali (Fig. 3). Alkali hydrolyzes the ester groups and opens the carbocyclic ring with the formation of chlorin e_6 (Fig. 3); acid, on the other hand, in addition to hydrolysis of the ester groups, removes carbon dioxide from the carbomethoxyl group, leading to phylloerythrin. Fischer concluded that the carbocyclic ring in the

[34] Fischer, Heckmaier, and Hagert, Ann., 505, 209 (1933).
[35] Fischer and Heckmaier, Ann., 508, 250 (1934); Fischer, Heckmaier, and Scherer, Ann., 510, 169 (1934).
[36] Fischer and Scherer, Ann., 519, 234 (1935).
[37] Stoll and Wiedemann, Helv. Chim. Acta, 16, 739 (1933); 17, 163 (1934).

pheophorbides (and hence in chlorophyll) is identical with that of the derived chlorophyll porphyrins such as phylloerythrin.

The Phase Test and Allomerization. Willstätter found that a characteristic reaction of chlorophyll, pheophytin, and pheophorbide in ether solution obtained by the action of cold alcoholic alkali is the sequence of color changes first noted by Molisch (green, then yellow, reverting to green, in the a series). Conant [38] found that the first product of the "phase test" reaction is an unstable chlorin (Willstätter's phytochlorin g) which, on being allowed to stand, is converted into pheopurpurin 18 (or purpurin 18) (XXII), a substance with a vivid purple color in ether solution and of acid number 18. Immediate esterification of the reaction mixture converts the unstable chlorin into pheopurpurin-7 trimethyl ester.

This phase test reaction was shown by Conant [39] to be an oxidative hydrolysis, with atmospheric oxygen acting as hydrogen acceptor. Hot saponification of either pheopurpurin 7 or its ester gives rise to a new chlorin,[40, 19] chlorin f (XXIV) (which Fischer later prepared and called rhodochlorin). Reduction of this dibasic acid with hydrogen iodide in acetic acid, and subsequent reoxidation in air, results in the formation of the dibasic acid, rhodoporphyrin (II). Since the latter contains no side chain on the γ-bridge carbon atom, neither does rhodochlorin. Rhodochlorin therefore contains a carboxyl group in the 6-position, and a propionic acid residue in the 7-position, according to the partial formula (Fig. 7). Pheopurpurin 7 (XXIII) contains in addition an α-keto (or glyoxalic acid) residue on the γ-bridge carbon atom.[40, 41] The unstable chlorin is a tricarboxylic acid or its lactone.[42, 43] Its γ-monomethyl ester has also been obtained using milder phase test conditions,[19, 44] which leave the 10-carbomethoxyl residue intact. Oxidation of pheophorbide a with pyridine-permanganate gives the same monomethyl chlorin g [42] (XXI). The oxidative hydrolysis of the phase test is therefore an oxidation on C_{10} (compare 10-hydroxypheophorbide a [XVII]) with hydrolysis of the carbocyclic ring at this point. Chlorin e_6 triester (XV), but not the non-methylated free chlorin e, gives the same result. Fischer's explanation is that the first reaction on the triester is ring closure to methyl pheophorbide a, which can take place only when the 6-carboxyl group is esterified. Pheopurpurin 18 (XXII) appears to be

[38] Conant and Moyer, J. Am. Chem. Soc., 52, 3013 (1930).
[39] Conant, Kamerling, and Steele, ibid., 53, 1615 (1931); Steele, ibid., 53, 3171 (1931).
[40] Conant, Hyde, Moyer, and Dietz, ibid., 53, 359 (1931).
[41] Dietz and Ross, ibid., 56, 159 (1934).
[42] Fischer and Kahr, Ann., 531, 209 (1937).
[43] Fischer and Conrad, Ann., 538, 143 (1939).
[44] Conant and Dietz, J. Am. Chem. Soc., 55, 839 (1933).

an anhydride; here the group on the γ-carbon atom has been further split, leaving a γ-carboxyl group, which undergoes anhydrization with the 6-carboxyl group.
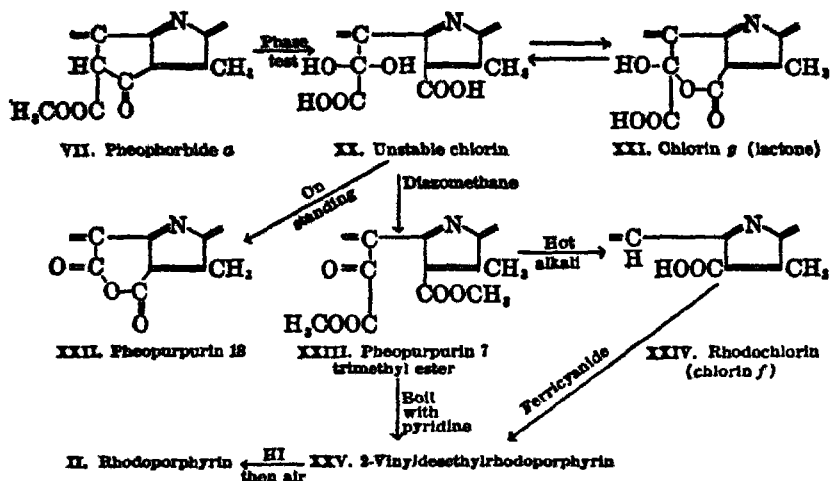
FIG. 7.—The phase test.

Direct dehydrogenation of rhodochlorin, using ferricyanide in an alkaline solution at room temperature,[40] gives not rhodoporphyrin but 2-vinyldesethylrhodoporphyrin (Conant's "isorhodoporphyrin"; see p. 1305), which differs from rhodochlorin by containing two hydrogen atoms less. 2-Vinyldesethylrhodoporphyrin is readily converted into rhodoporphyrin by reduction with hydrogen iodide, followed by atmospheric oxidation.

Willstätter described a reaction of chlorophyll which he termed allomerization. When an alcoholic solution of chlorophyll is allowed to stand for some time, the resulting material, "allomerized chlorophyll," is no longer capable of giving the phase test; from the alcoholic solution he prepared (phyto)chlorin g. Conant[40] showed that allomerized chlorophyll, on hydrolysis, removal of magnesium by acid, and esterification with diazomethane, gives the same product—pheopurpurin-7 trimethyl ester—as does the phase test. This is in contrast to unallomerized chlorophyll, which under identical treatment yields the trimethyl ester of chlorin e_6. That allomerization is an oxidative reaction was shown by Conant[39] using a modified Warburg apparatus; two equivalents of oxygen are absorbed per mole of chlorophyll. He further found that chlorophyll and the pheophorbides can be dehydrogenated,[15] using two equivalents of potassium molybdicyanide, and again pheo-

purpurin-7 ester is obtained. Hence the phase test, allomerization, and direct dehydrogenation all lead through pheopurpurin-7 ester and rhodochlorin to either 2-vinyldesethylrhodoporphyrin or to rhodoporphyrin, while rapid saponification, which excludes oxidation, results in chlorin e_6, from which phylloporphyrin is derived. Hence, according to Conant,[45] the fundamental nuclear structure of the pheophorbides and of chlorophyll a is that of rhodochlorin, which is a 2-vinyldesethyldihydrorhodoporphyrin.

The Vinyl Group. Conant's view of the chlorophyll nucleus as a dihydroporphyrin, and Fischer's view, based on the isomerization with hydrogen iodide, that it was an isomerized porphyrin, were reconciled by the later work of Fischer. He established that chlorophyll, the phorbides, chlorins, and purpurins contained (a) an unsaturated side chain, viz., the vinyl group in the 2-position (Figs. 1 and 11), and (b) two "extra hydrogen atoms" on the porphyrin nucleus. If pheophorbide is hydrogenated with addition of three moles of hydrogen, a leuco compound is formed, which upon oxidation in air in an acid medium is converted into pheoporphyrin a_5 (XI), whereas in a neutral medium, or upon partial hydrogenation with platinum oxide or palladium, where only one mole of hydrogen is taken up, dihydropheophorbide a (mesopheophorbide a) is obtained.[46, 47] Chlorin e_6 and purpurin 7 also give dihydro (meso) compounds; spectroscopically they differ very slightly from the parent substance—an indication that the unsaturated group involved is not part of the nuclear structure. Fischer uses the general term "meso" for compounds in which the vinyl group has been reduced to ethyl.

Secondly, if the hydrogen iodide reaction is carried out in the cold, a series of ketoporphyrins is produced.[45, 48] For instance, chlorophyll a and pheophorbide a both yield oxopheoporphyrin a_5 monomethyl ester ("isopheoporphyrin a_6" in the older literature), while pyropheophorbide yields oxophylloerythrin. These two new compounds contain two keto groups each. On heating oxophylloerythrin an oxorhodoporphyrin is formed; the Wolff-Kishner reaction converts this to normal rhodoporphyrin, hence the oxo group is an acetyl residue ($-COCH_3 \rightarrow -CH_2CH_3$). The position of this residue and therefore of the parent unsaturated group was established by the fact that oxophylloerythrin heated in a sealed tube with concentrated hydrochloric acid (which replaces the acetyl group by hydrogen), followed by esterification, gave a desethylphylloerythrin and a desethylpyrroporphyrin. The latter

[44] Conant and Bailey, ibid., 55, 795 (1933).
[45] Fischer and Lakatos, Ann., 506, 123 (1933).
[47] Stoll and Wiedemann, Naturwissenschaften, 20, 791 (1932)
[48] Fischer and Riedmair, Ann., 506, 87 (1933).

was identical with a synthetic 2-desethylpyrroporphyrin. Also the desethylphylloerythrin was reduced to a desethyldesoxophylloerythrin, and this was identical with one of the two possible synthetic compounds, viz., the 2-desethyl.[49] Hence the unsaturated group in chlorophyll is in the 2-position.

The third reaction, that of diazoacetic ester, proved that the unsaturated group is a vinyl residue; this will produce an acetyl group on oxidation (cf. hemin and protoporphyrin, each of which contains two vinyl groups [p. 1284, Fig. 41]). Diazoacetic ester adds to the vinyl group with evolution of nitrogen, and, on drastic oxidation of the addition compound with chromic acid, one of the products isolated was methylmaleimidecyclopropylcarboxylic acid (XXVIII).[50]
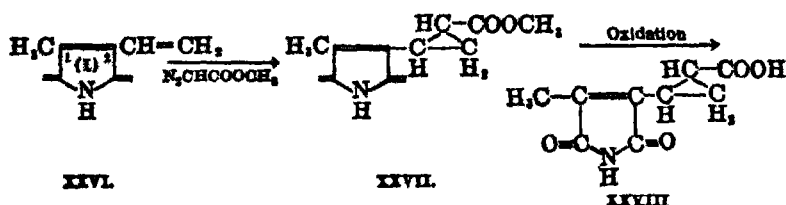
FIG. 8.—The diazoacetic ester reaction.

The pheophorbides, the chlorins derived from chlorophyll by degradation, the purpurins, and 2-vinylrhodoporphyrin add diazoacetic ester, and therefore contain the vinyl group. The oxo reaction is easily explained: hydrogen iodide adds to the vinyl group, hydrolysis occurs with replacement of iodine by hydroxyl, and spontaneous dehydrogenation takes place, giving the acetyl residue.
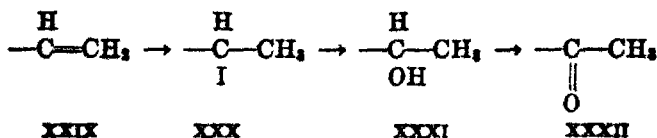
FIG. 9.—The oxo reaction (a series).

The Dihydroporphyrin Nucleus. Since pheoporphyrin a_5 is isomeric with pheophorbide a, the hydrogen iodide isomerization must be in effect a migration of two hydrogen atoms from somewhere in the nucleus to the vinyl group. The porphyrins which result contain an ethyl residue in the 2-position. The position of these two "extra hydrogen atoms" remained to be determined. Fischer showed that in chlorin a_6 trimethyl ester the extra hydrogens can be replaced by hydroxyl

[49] Fischer and Rose, Ann., 519, 1 (1935).
[50] Fischer and Medick, Ann., 517, 245 (1935).

groups, giving dihydroxychlorin e_6 trimethyl ester.[51] This was achieved by oxidizing chlorin e_6 trimethyl ester with silver oxide in pyridine-methanol-dioxan. Mesochlorin e_6 triester and the diazoacetic derivative of chlorin e_6 triester give corresponding derivatives. Purpurin 7 also gives a dihydroxy derivative. The structure of these is most simply explained if the extra hydrogen atoms are in positions 5 and 6 in nucleus III or 7 and 8 in nucleus IV. Fischer examined chlorophyll derivatives for optical activity, and not only chlorophyll but also the simple chlorins, rhodo-, phyllo-, and pyrrochlorins are optically active. The corresponding isomeric porphyrins are inactive. This work gave no conclusive evidence in favor of either nucleus.[52]

The products of oxidative degradation of chlorophyll derivatives were then studied. Küster found hematinic acid and ethylmethylmaleimide (p. 1295). Later work showed that β-methylpyrrole nuclei with a free β'-position (e.g., pyrroporphyrin [III]), in which the 6-position of nucleus III is unsubstituted), gave in addition citraconimide.[53] Phyllochlorin (XXXIII) in the oxidation described above would be expected to give all three degradation products, but in fact gave instead of inactive hematinic acid an optically active "acid fraction."[54] Oxidation of pheophorbide a and b, mesopheophorbide a, and purpurin 7 gave the same result. From this "acid fraction" were isolated optically active hemotricarboxylic imide (i.e., a dihydrohematinic acid) and traces of hematinic acid.

XXXIII. Phyllochlorin.   XXXIV. Hemotricarboxylimide.
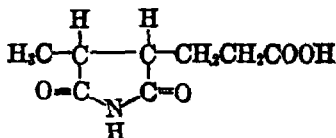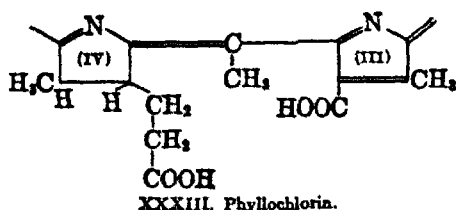
FIG. 10

This hemotricarboxylic imide can come only from nucleus IV, carrying the propionic acid side chain. The asymmetry of phyllochlorin is due solely to the extra hydrogen atoms (pheophorbide a, for instance, has another asymmetric center in position 10), hence these extra hydrogen atoms must be in nucleus IV to give an optically active hemotricarboxylic imide. Fischer therefore assigns the two extra hydrogen atoms to nucleus IV in positions 7 and 8.

[51] Fischer and Lautsch, Ann., 528, 247 (1937).
[52] Summarised by Steele, Chem. Rev., 20, 1 (1937); see also Fischer et al., Ann., 534, 292 (1938).
[53] Fischer and Breitner, Ann., 522, 155 (1936).
[54] Fischer and Wenderoth, Ann., 537, 170 (1939); 545, 140 (1940).

Depending on the resulting arrangement of the double bonds in the porphyrin system, the formula for chlorophyll a is therefore XXXV or XXXVI. Fischer prefers formula XXXV as here the carbocyclic ring
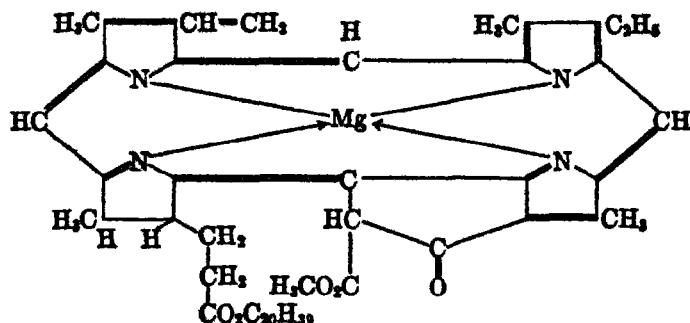
XXXV. Fischer's structure
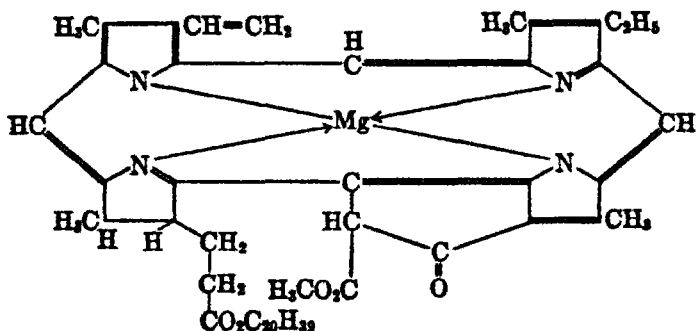
XXXVI. Alternative structure

Fig. 11.—Chlorophyll a.

becomes less stable on enolization and therefore more in accord with the experimental results, e.g., the very easy conversion to chlorin e_0 or to the phase test products. On the contrary, enolization of XXXVI would give a carbocyclic ring with two double bonds, which would tend to stabilize the system. All the formulas in this chapter have been based on the arrangement of double bonds in XXXV, but it must be remembered that in the porphyrins there is no definite proof that the double bonds are fixed (p. 1288).

CHLOROPHYLL *b*

The Formyl Group. Chlorophyll *b* differs from *a* in that it contains an oxygen atom in place of two hydrogen atoms.[3] Conant [55] obtained from methylpheophorbide *b*, by pyrolysis, phase test reaction, and saponification, a series of compounds entirely parallel to those in the *a* series (Fig. 7). These rhodins, corresponding to the chlorins in the *a* series, still retained the extra oxygen atom and formed semicarbazones, indicating the presence of a carbonyl group. Warburg [56] also obtained carbonyl derivatives in the *b* series, while Stoll and Wiedemann isolated a dioxime of pheophorbide *b*.[57]

Fischer,[58] using the hydrogen iodide reaction, showed that pheophorbide *b* and rhodin g_7 (analogous to chlorin e_6) gave rise to porphyrins, viz., the pheoporphyrin *b* series and the rhodinporphyrin *g* series, corresponding respectively to the pheoporphyrin *a* and the chloroporphyrin *e* compounds of the *a* series (Fig. 5). Hence pheophorbide *b* contains the carbocyclic ring of the *a* series. Pheophorbide *b* forms an acetal and also an oxynitrile derivative, while the carbonyl group in the carbocyclic ring (position 9) is incapable of such reactions. Further, rhodin g_7, in which the carbocyclic ring is absent, can still form an oxime, indicating that the new carbonyl group is in some other part of the molecule.[59, 55]

Chlorophyll *b* and the rhodins contain the vinyl group in the 2-position, with which diazoacetic ester gives addition products as in the *a* series. Hydrogen iodide on rhodin g_7 trimethyl ester gives the trimethyl ester of rhodinporphyrin g_7. This corresponds to chloroporphyin e_6 (XIV) and has the vinyl group saturated to an ethyl group with the extra hydrogen atoms. This compound forms a monoöxime, hence still contains the carbonyl group of the *b* series. Hydrogen iodide in the cold (oxo reaction of the *a* series) gives rhodinporphyrin g_8 (Fig. 12), which no longer forms an oxime but contains an additional carboxyl group. Hence the parent compound has a formyl group. Hydrogen bromide or chloride at high temperatures removes the carboxyl group and gives rise to 3-desmethylphylloporphyrin and 3-desmethylpyrroporphyrin. Hence the formyl group is in the 3-position, replacing a methyl group of the *a* series.[60]

Further proof was given by the synthesis from substituted pyrroles of

[55] Conant, Dietz, and Werner, *J. Am. Chem. Soc.*, **53**, 4436 (1931).

[56] Warburg and Christian, *Biochem. Z.*, **235**, 240 (1931); Warburg and Negelein, *ibid.*, **244**, 9 (1932).

[57] Stoll and Wiedemann, *Helv. Chim. Acta*, **17**, 456 (1934).

[58] Fischer, Hendschel, and Nüssler, *Ann.*, **506**, 83 (1933).

[59] Fischer, Breitner, Hendschel, and Nüssler, *Ann.*, **503**, 1 (1933).

[60] Fischer and Breitner, *Ann.*, **510**, 183 (1934).

3-desmethyldesoxophylloerythrin.[49] This compound is obtained from the ester of rhodinporphyrin g_8 by reduction, whereby the 3-carboxyl group is reduced to a hydrogen atom, and again ring closure takes place between the γ- and 6-positions. Alternately, pyrolysis of rhodinporphyrin g_8 in pyridine effects ring closure with formation of pheoporphyrin b_7 (cf. e_6 to a_5, Fig. 5). If the oxime (on the 9-carbonyl) of b_7 is heated with hydrogen bromide, it loses two carboxyl groups, and 3-desmethyldesoxophylloerythrin again results.[61] Hence chlorophyll b is 3-formyl-desmethylchlorophyll a.
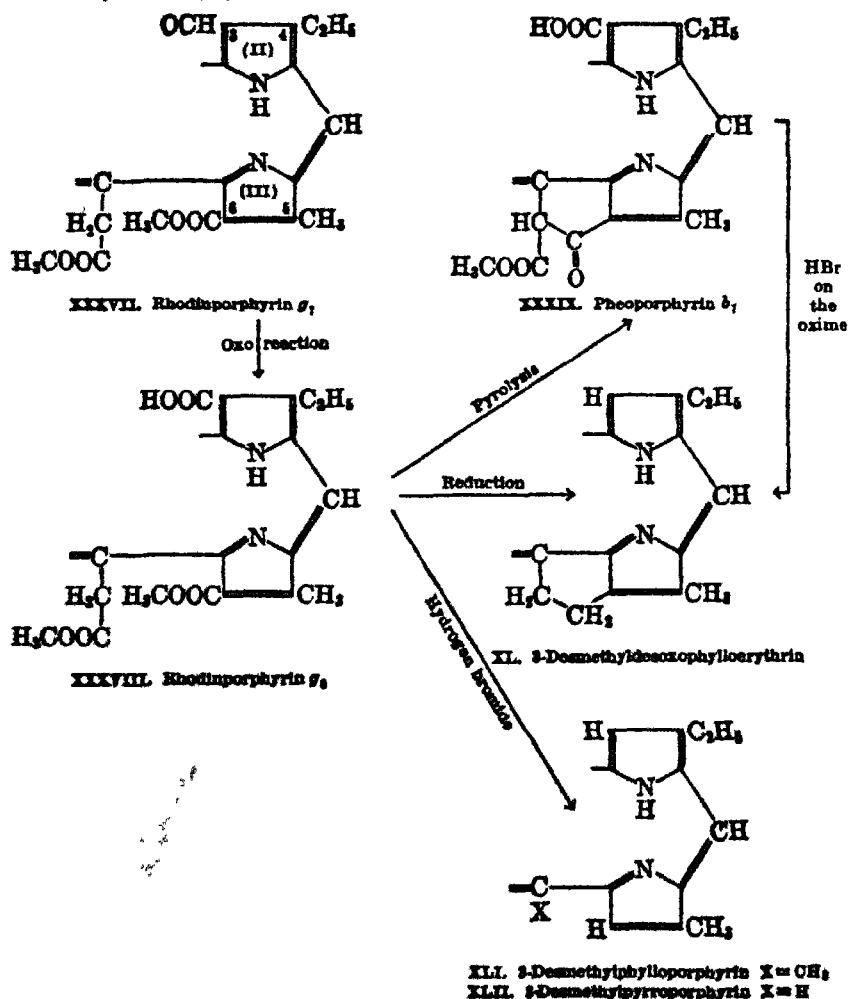
FIG. 12.—Proof of the formyl group.

[61] Fischer and Breitner, Ann., 516, 61 (1935).

The 3-formyl group can be reduced to the methanol group without affecting the vinyl group by the use of aluminum isopropoxide. Methylpheophorbide b gives methylpheophorbide b-3-methanol (—CH_2OH replaces —CHO in position 3).[42]

The conversion of the b into the a series has been accomplished by catalytic reduction in formic acid of pheoporphyrin b_6, the products being pheoporphyrin a_5 and its reduction product, 9-hydroxydesoxopheoporphyrin a_5.[43]

PARTIAL SYNTHESIS OF CHLOROPHYLL

Total Synthesis of Pheoporphyrin a_5. Phylloerythrin (XII), which contains both the porphyrin system and the carbocyclic ring, has been prepared synthetically. To convert this to pheophorbide a requires: (a) addition of the carbomethoxyl residue to the carbocyclic ring; (b) reduction of the porphyrin system to the phorbide by the addition of the two extra hydrogen atoms; and (c) the oxidation of the ethyl group to the vinyl in position 2. Since, however, the oxidation of this ethyl group has so far been found impossible in such a complex molecule, two parallel series of syntheses have been attempted. The first, among the meso compounds, ignores the problem of the vinyl group, and is concerned with (a) and (b).

(a) The introduction of the carbomethoxyl residue to phylloerythrin would give pheoporphyrin a_5 (XI), but attempts to do this have met with failure. Synthesis of pheoporphyrin a_5 has, however, been achieved from phylloporphyrin (IV), which has itself been synthesized from substituted pyrroles. Phylloporphyrin contains a γ-methyl group (—CH_3). This was oxidized to the formyl residue (—CHO), and HCN adds to the resulting γ-formylpyrroporphyrin methyl ester to give pyrroporphyrin γ-cyanhydrin methyl ester (—HCOHC:N).[44] This, on being allowed to stand with concentrated sulfuric acid, is partly saponified to the acid amide (—HCOHCONH$_2$). Reduction with a platinum oxide catalyst in formic acid gives the acid amide of isochloroporphyrin e_4, the free porphyrin being obtained by hydrolysis with hydrochloric acid (—CH_2COOH).[45] This porphyrin had been previously prepared analytically from chlorin e_6. Esterification of the latter with methyl alcohol and hydrochloric acid gives a diester in which the carboxyl group in the 6-position is free. This chlorin e_6 dimethyl ester on pyrolysis loses the

[42] Fischer, Mittenzwei, and Hevér, Ann., **545**, 154 (1940).
[43] Fischer and Grassl, Ann., **517**, 1 (1935).
[44] Fischer and Stier, Ann., **542**, 224 (1939).
[45] Fischer, Kanngiesser, and Stier, Naturwissenschaften, **28**, 30 (1940); Ann., **542**, 258 (1940).

carboxyl group to give isochlorin e_4 dimethyl ester, and catalytic reduction to the leuco compound, followed by reoxidation in air, gives rise to the corresponding (and isomeric) porphyrin, isochloroporphyrin e_4 dimethyl ester (XLIII).[66]

Fischer found means of introducing the formyl group into porphyrins by treating the hemin (complex iron salt) of the porphyrin with unsymmetrical dichloromethyl ether in the presence of stannic chloride or bromide. In the case of isochloroporphyrin e_4 ester the formyl group enters at position 6, and ring closure with the γ-acetic ester side chain takes place spontaneously, so that the product isolated is 9-hydroxydesoxopheoporphyrin a_5. Oxidation with chromic acid in acetic acid gives pheoporphyrin a_5.[67]

XLIII. Isochloroporphyrin e_4 ester   XLIV.

XLV. 9-Hydroxydesoxopheoporphyrin a_5 XL. Pheoporphyrin a_5
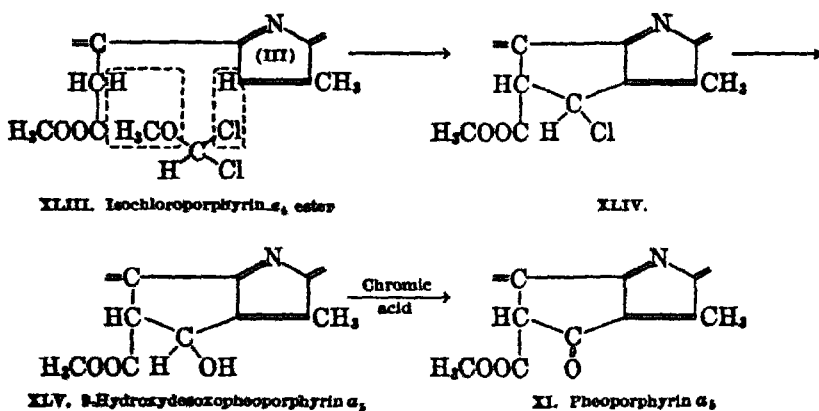
Fig. 13.—Synthesis of Pheoporphyrin a_5.

(b) **Synthetic Chlorins.** The introduction of the two extra hydrogen atoms constitutes a synthesis of mesochlorins from the chlorophyll porphyrins. Synthetic chlorins have been prepared by several methods. Fischer [68] used sodium and amyl alcohol on porphyrin-iron salts; Treibs and Wiedemann [69] and Fischer [70] used catalytic hydrogenation of porphyrins. Pyrroporphyrin gives a mesopyrrochlorin, and a mesophyllochlorin has also been prepared. The absolute identity of these compounds with the corresponding substances prepared analytically has not been established, and in any event the methods of preparation give no proof of the position of the extra hydrogen atoms which have been introduced.

[66] Fischer and Kellermann, *Ann.*, **519**, 209 (1935).
[67] Fischer and Kellermann, *Ann.*, **524**, 25 (1936).
[68] Fischer and Halberger, *Ann.*, **471**, 285 (1929).
[69] Treibs and Wiedemann, *Ann.*, **471**, 215 (1929).
[70] Fischer and Lakatos, *Ann.*, **506**, 138 (1933); Fischer and Herrle, *Ann.*, **530**, 230 (1937).

The next problem is the synthesis of the parallel series of compounds from a substance already containing the vinyl group, e.g., a vinyldesethylporphyrin. Several vinyldesethylporphyrins have been prepared analytically. As an alternative there is the possibility of the synthesis of chlorins directly from substances simpler than porphyrins, probably the only method that would give absolute proof of the position of the extra hydrogen atoms.

Introduction of the Phytyl Group and of Magnesium. Willstätter [71] introduced the phytyl group into pheophorbide biologically by reversing the action of the chlorophyllase enzyme. Using phosgene, Fischer [72] found it possible to esterify pheophorbide *a* with various alcohols of high molecular weight; these included phytol, geraniol, menthol, and cetyl alcohol. Thus a synthesis of pheophytin from pheophorbide was achieved, and the synthetic product was in all its properties identical with natural pheophytin.

Willstätter [73] prepared chlorophyll from pheophytin by introducing magnesium through the medium of the Grignard reagent. Fischer improved the method and converted methylpheophorbide *a* into chlorophyllide *a*,[74] ethylpheophorbide *b* into ethylchlorophyllide *b*,[75] pheoporphyrin a_5 into pheoporphyrin a_5-phyllin, and pheophytin *a* into chlorophyll *a*.[76]

BACTERIOCHLOROPHYLL AND PROTOCHLOROPHYLL

Nature appears to produce several pigments similar to chlorophyll. Bacteriochlorophyll is the assimilatory pigment of the photosynthetic purple and brown bacteria. It differs from chlorophyll *a* in the 2-position, where an acetyl residue replaces the vinyl group, and it also contains two hydrogen atoms more than the phorbide system, i.e., it is based on a dihydrophorbide or tetrahydroporphyrin nucleus. These two new hydrogen atoms are probably in nucleus II in positions 3 and 4 (and therefore based on chlorophyll structure XXXVI), and they are easily removed by dehydrogenation. The resultant product after removal of magnesium and phytol is 2-desvinyl-2-acetylpheophorbide *a* (dehydrobacteriopheophorbide). This compound has also been obtained from the chlorophyll *a* derivative, chlorin e_6, by synthesis.[77, 78] The relationship can be summarized as follows: [63]

[71] Willstätter and Benz, *Ann.*, **358**, 267 (1908); Willstätter and Stoll, *Ann.*, **380**, 148 (1911).

[72] Fischer and Schmidt, *Ann.*, **519**, 244 (1935).

[73] Willstätter and Forsen, *Ann.*, **396**, 180 (1913).

[74] Fischer and Spielberger, *Ann.*, **510**, 156 (1934).

[75] Fischer and Spielberger, *Ann.*, **515**, 130 (1935).

[76] Fischer and Goebel, *Ann.*, **524**, 269 (1936).

[77] Fischer, Lautsch, and Lin, *Ann.*, **534**, 1 (1938).

[78] Fischer, Oestreicher, and Albert, *Ann.*, **538**, 128 (1939).

Pheophorbide *a* ⇌ chlorin *e₆* ⇌ 2,*α*-hydroxychlorin *e₆* ⇌
2-acetylchlorin *e₆* ⇌ 2-desvinyl-2-acetylpheophorbide *a*
(dehydrobacteriopheophorbide)
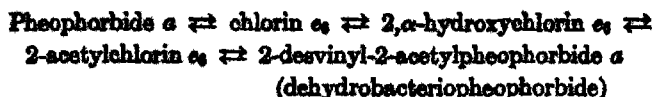
Protochlorophyll is present in pumpkin seeds and the rinds of gourds. Noack and Kiessling [79] showed that it was a porphyrin. Stoll and Wiedemann suggested and Fischer [78, 80] established that it is a derivative of pheoporphyrin a_5 (XI), viz., the phyllin (i.e., the magnesium salt) of 2-vinyldesethylpheoporphyrin a_5 phytyl ester. Protochlorophyll is optically inactive although it contains a potential asymmetric center at C_{10}. This is additional proof that the asymmetry of chlorophyll resides in the 7-, 8-positions.

A complete solution of the exact structure of the chlorophyll molecule would further the elucidation of the photosynthetic process in plants and also of the newly developing field of chlorophyll therapy. Chlorophyll may act in photosynthesis in two ways: (1) as a pigment, absorbing solar energy in the form of certain wavelengths of light; and (2) as a chemical compound. The possibility of (2) entering into the photosynthetic process depends on: (*a*) the reactivity of the molecule, e.g., reactive hydrogens and the ability of the magnesium to form salts; and (*b*) the physical state of the chlorophyll, i.e., whether it is in colloidal form or adsorbed on colloidal surfaces. A final solution of the molecular structure of chlorophyll will help to solve (*a*) and may give indications of the relationship or interconversion (if any) between chlorophylls *a* and *b* in the plant. Chlorophyll and hemin are closely related. Chlorophyll breaks down in the animal digestive tract to porphyrins, and attempts have been made to cure anemia (due to lack of pigment) with chlorophyll and its derived porphyrins. Again, recent medical research has shown that chlorophyll in contact with living tissues (and therefore in colloidal form?) is a bactericide; this may be directly related to its ability in plant tissues to break down carbon dioxide with the release of oxygen.

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FISCHER, *Naturwissenschaften*, **28**, 401 (1940.)

  [79] Noack and Kiessling, *Z. physiol. Chem.*, **182**, 13 (1929); **193**, 97 (1930).

  [80] Fischer and Oestreicher, *ibid.*, **262**, 243 (1940).

CHAPTER 18

THE ANTHOCYANINS AND THE FLAVONES

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CONTENTS

THE ANTHOCYANINS

Introduction. The pigments of plants are roughly divisible into two major classes.[1, 2, 3, 4, 5] The plastid pigments represent one group.[4] They are associated with the protoplasmic structure of the plant. The second group consists of those pigments which generally exist in solution in the cell sap. These pigments belong to a group of glycosides designated as "anthocyanins."[3, 4, *] The innumerable shades of blue, purple, violet, mauve, and magenta and nearly all the reds which appear in flowers, fruits, leaves, and stems of plants are due to anthocyanin pigments. The sugar-free pigments or aglycons are called *anthocyanidins*.[4] Although it is held that the anthocyanins are usually dissolved in the cell sap, they can also occur in an amorphous or crystalline state as in the *Delphinium* spp., *Passiflora* spp., *Rubus* spp., and others.

The pioneer researches of Willstätter and his students [6, 7, 8] made it clear that the numerous individual anthocyanins contain similar nuclei. The wide variations in color are due to slight alterations in the molecule which do not affect the basic molecular skeleton. Thus, the anthocyanins represent a chemical class of natural products, in the same sense as the fats, carbohydrates, or proteins represent distinct classes.

The Basic Structure of the Anthocyanins. Willstätter's success [6, 7] with these plant coloring matters was to a large extent due to his early

[1] Rupe, "Die Chemie der natürlichen Farbstoffe," Vieweg und Sohn, Braunschweig (1900), Vol. I; (1909), Vol. II.

[2] Mayer, "Chemie der organischen Farbstoffe," 3rd ed., Springer, Berlin (1935), Vol. II, pp. 134–150.

[3] Perkin and Everest, "The Natural Organic Colouring Matters," Longmans, Green and Co., London (1918).

[4] Wheldale, "The Anthocyanin Pigments of Plants," University Press, Cambridge (1916).

[5] Karrer, "Handbuch der Pflanzenanalyse," edited by Klein, Springer, Vienna (1932), Vol. III, pp. 941–984.

[*] The term "anthocyan" is derived from the Greek roots signifying respectively "flower" and "blue." It was introduced by the botanist Marquart in 1835 to designate the blue pigments of flowers. Shortly thereafter the belief developed that the red and blue pigments were merely different forms of the same substance and that their different colors were due to variations in the character of the cell sap; consequently, the use of the term was extended to include all the soluble pigments of this group. When it was learned that these pigments are always combined with sugars, and thus occur as glycosides, the ending "in" was attached.

[6] Willstätter, *Sitzber. preuss. Akad. Wiss.*, 29, 402, 769 (1914); also *Ber.*, 47, 2865 (1914).

[7] Willstätter and co-workers, *Ann.*, 401, 189 (1913); 408, 1, 15, 42, 61, 83, 110, 147 (1915); 412, 113, 137, 149, 164, 178, 195, 217, 231 (1916); *Ber.*, 57, 1938, 1945 (1924).

[8] Robinson, *Naturwissenschaften*, 20, 612 (1932). Summary of Professor R. Willstätter's investigations on the anthocyanins.

recognition of their amphoteric nature. They are capable of forming salts with both acids and bases. The salts formed with acids were first recognized as oxonium salts of the type known as flavylium salts. The methods of isolation and purification employed with these pigments rest on this basis.

The fundamental parent substance of the entire group is the heterocyclic nucleus known as benzopyrylium chloride (I) discovered by Decker and von Fellenberg[9] which they formulated on the basis of the oxonium theory.[*] By substituting a phenyl residue in position 2 of the benzopyrylium chloride (I), 2-phenylbenzopyrylium chloride or flavylium chloride (II) is obtained. The placement of hydroxyl groups in positions 3, 5, and 7 yields 3,5,7-trihydroxyflavylium chloride (III), the simplest intact structural unit of the anthocyanins. In fact,

[*] Decker and von Fellenberg, *Ann.*, **364**, 1 (1908).

[†] The properties and methods for the synthesis of benzopyrylium salts have been reviewed by Hill [*Chem. Rev.*, **19**, 27 (1936)]. Both Hill and Dilthey and coworkers [*J. prakt. Chem.*, **131**, 1 (1931); **138**, 42 (1933); *Ber.*, **64**, 2082 (1931)] have pointed out the disadvantages of the oxonium salt theory and have suggested that the compounds are carbonium or carbenium salts.

Recent work by Shriner and Moffett [*J. Am. Chem. Soc.*, **61**, 1474 (1939); **62**, 2711 (1940); **63**, 1694 (1941)] has shown that benzopyrylium salts differ markedly from the true oxonium salts of ethers and γ-pyrones. They resemble the carbonium salts derived from triarylcarbinols. All the properties, degradation products, and syntheses strongly suggest that carbon atoms 2, 3, and 4 of the benzopyrylium salts constitute an allylic system:
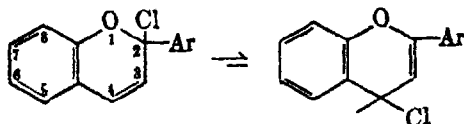
The activity of the chlorine is further enhanced by the fact that the first of these structures is an α-chloroether and the second is a vinylog of an α-chloroether. Conductivity measurements show that in nitrobenzene solutions the chlorides are ionized to about 3 to 10 per cent and that the perchlorates are about 60 to 80 per cent dissociated. Hence the salts represent a special case of the allylic system in which the chief resonating carbonium ions are:
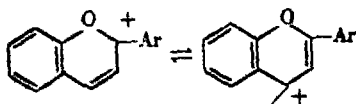
The anions may be Cl⁻, Br⁻, I⁻, $(ClO_4)^-$, or $(FeCl_4)^-$. The anthocyanidins which are polyhydroxy (and -methoxy) flavylium salts contain the above structures but because of the presence of the hydroxyl groups may tautomerize to a quinoid structure in a manner similar to that shown by the hydroxytriphenylmethane dyes of the aurin type. This viewpoint was presented by Professor Shriner at the Ninth Organic Chemistry Symposium, Ann Arbor, Michigan (1941).

all the members of this group found to date can be regarded as poly-
hydroxy-2-phenylbenzopyrylium salts.

I	II	III
Benzopyrylium chloride	2-Phenylbenzopyrylium chloride (flavylium chloride)	3,5,1-Trihydroxy-flavylium chloride
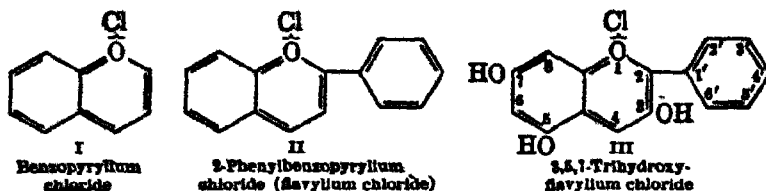
The Type Group of the Anthocyanidins. The investigations of
Willstätter,[6, 7, 8] Karrer,[9] and Robinson [9, 10, 11, 12, 13] have shown that
there are six type groups of the anthocyanins to which the various indi-
viduals can be referred.* These groups are known respectively as
pelargonidin (IV), cyanidin (V), delphinidin (VI), peonidin (VII),
malvidin† or syringidin (VIII), and hirsutidin (IX). It is to be
observed that pelargonidin (IV), cyanidin (V), and delphinidin (VI) are
the fundamental types of the class, whereas peonidin (VII) is a mono-
methyl ether of cyanidin, and malvidin (syringidin) (VIII) and hirsutidin
(IX) are respectively the di- and trimethyl ethers of delphinidin. All
the type groups have been synthesized by Robinson and his co-workers
through methods that leave no doubt as to the validity of their
structure.‡

IV	V
Pelargonidin [3,5,7,4'-tetrahydroxy-2-phenylbenzopyrylium chloride]	Cyanidin [3,5,7,3',4'-pentahydroxy-2-phenylbenzopyrylium chloride]
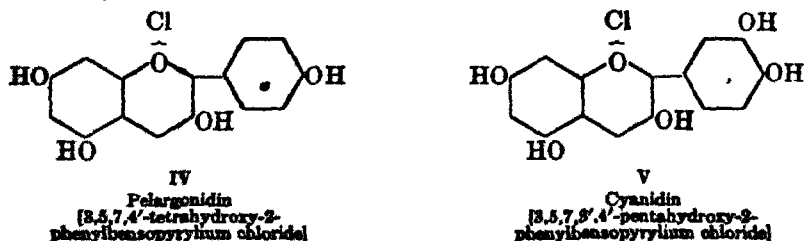
[10] Robinson, *Nature*, 127, 94 (1936); *Ber.*, 67A, 85 (1934).

[11] Robinson, *Nature* (Royal Jubilee Number), 135, 732 (1935).

[12] Robinson, *J. Soc. Chem. Ind.*, 52, 737 (1933).

[13] Robinson, President's Address, Section B, Chemistry, British Association for the
Advancement of Science; reprinted in *Nature*, 132, 625 (1933).

* The classes are usually designated by root names derived from the Latin botanical
nomenclature.

† Malvidin is also called syringidin since it yields syringic acid on degradation with
dilute alkali.

‡ It is a remarkable fact that almost the whole range of anthocyanin pigments of
flowers, fruits, and blossoms is derived from the three fundamental anthocyanidin types,
IV, V, VI, by various substitutions in the hydroxyl group. However, some excep-
tions exist. The bluest anthocyanins in the beet, for instance, originally isolated by
Willstätter, are nitrogenous pigments (see Robinson and Robinson, *J. Chem. Soc.*, 1439
[1932]; 25 [1933]; 446, 449, 453 [1937]).
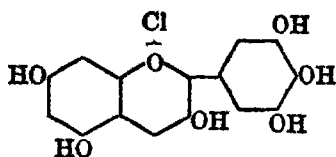
VI
Delphinidin
[3,5,7,3',4',5'-hexahydroxy-2-
phenylbenzopyrylium chloride]
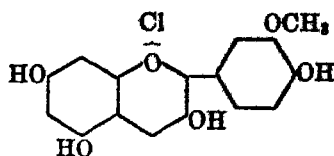
VII
Peonidin
[3,5,7,4'-tetrahydroxy-3'-methoxy-
2-phenylbenzopyrylium chloride]
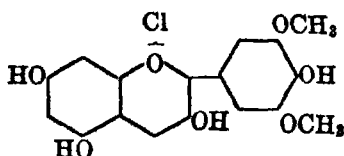
VIII
Malvidin (syringidin)
[3,5,7,4'-tetrahydroxy-3',5'-
dimethoxy-2-phenylbenzo-
pyrylium chloride]
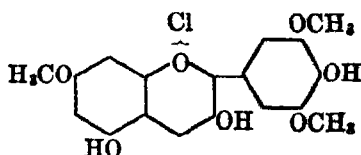
IX
Hirsutidin
[3,5,4'-trihydroxy-7,3',5'-tri-
methoxy-2-phenylbenzopyrylium
chloride]

The Glycosidic Nature of the Anthocyanins.[5, 8, 11, 12, 13] All the members isolated so far yield in addition to the *anthocyanidin* a sugar (p. 1572), or several sugars, when boiled for a short time with dilute mineral acids. The greater number of the anthocyanins fall into a comparatively restricted number of categories with the sugar residues attached to the 3- or 3,5-hydroxyls; thus (a) the 3-monoglucosides and 3-monogalactosides; (b) the 3-rhamnosides and other 3-pentosides; (c) the 3-biosides; (d) the 3,5-diglucosides; and (e) the acylated anthocyanins. The anthocyanins of class (d) are the most widely distributed and best-known members of the group. Pelargonin, the 3,5-diglucoside of pelargonidin (IV), the pigment of the scarlet pelargonium and possibly the first member of the soluble pigments to be obtained in a crystalline condition,[7, 8, 10] belongs to this group, as does cyanin, isolated by Willstätter in 1914 from the cornflower. Pelargonin, cyanin, peonin, malvin, and hirsutin have been synthesized by Robinson.[10] Certain anthocyanins, delphinin, gentianin, monardaein, and salvianin, for instance, yield, in addition to the pigment and the sugar or sugars, a third component which is invariably an organic acid. These represent the acylated anthocyanins [group (e) above]. The acids found so far are p-hydroxybenzoic, malonic, p-hydroxycinnamic, and p-coumaric acid (see the work of Karrer[5, 14]). The acid radicals can be either in ester combination with one of the hydroxyl groups of the pigment nucleus, or attached to an hydroxyl of the sugar component. The hydrolysis of

[14] Karrer and co-workers, *Helv. Chim. Acta.*, 10, 67, 729 (1927); 12, 292 (1929); 15, 507 (1932).

some representative anthocyanins is illustrated in the following equations:

$$C_{27}H_{31}O_{15}Cl + 2H_2O \rightarrow C_{15}H_{11}O_5Cl + 2C_6H_{12}O_6$$
Pelargonin chloride   Pelargonidin   Glucose
chloride

$$C_{36}H_{31}O_{16}Cl + 3H_2O \rightarrow C_{15}H_{11}O_7Cl + C_6H_{12}O_6 + C_9H_8O_3$$
Gentianin chloride   Delphinidin   Glucose   p-Coumaric
chloride   acid

$$C_{41}H_{39}O_{21}Cl + 4H_2O \rightarrow C_{15}H_{11}O_7Cl + 2C_6H_{12}O_6 + 2C_7H_6O_3$$
Delphinin chloride   Delphinidin   Glucose   p-Hydroxy-
chloride   benzoic acid
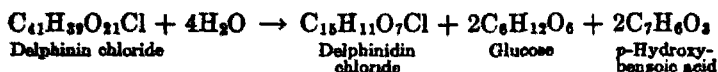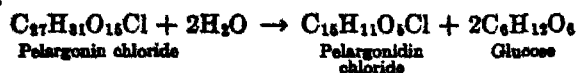
The Degradation Products of the Anthocyanidins.[5, 7, 14] The occurrence of the 2-phenylbenzopyrylium nucleus (II) in the various anthocyanins was originally established by Willstätter through an alkaline fusion of the sugar-free pigments.[*] When the empirical formulas of the three parent classes are compared, it becomes evident that they differ from each other by a single oxygen atom, as represented below:

Pelargonidin chloride $C_{15}H_{11}O_5Cl$ or $C_{15}H_7OCl(OH)_4$

Cyanidin chloride $C_{15}H_{11}O_6Cl$ or $C_{15}H_6OCl(OH)_5$

Delphinidin chloride $C_{15}H_{11}O_7Cl$ or $C_{15}H_5OCl(OH)_6$
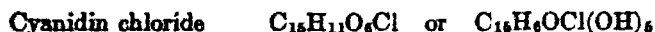
These three anthocyanidins degrade upon fusion with potassium hydroxide into two simple products, one of which is a phenol, the other a phenolcarboxylic acid. The phenol obtained from each of the three homologous anthocyanidins is the same, namely, phloroglucinol (1,3,5-trihydroxybenzene) (X). The phenolcarboxylic acid obtained from the simplest anthocyanidin, pelargonidin (IV), is p-hydroxybenzoic acid (XI); that from the next simplest, cyanidin (V) is 3,4-dihydroxybenzoic acid or protocatechuic acid (XII); that from the third, delphinidin (VI), is 3,4,5-trihydroxybenzoic acid or gallic acid (XIII).

The relationship of the phenol common to the three parent anthocyanidins and the respective phenolic acids is illustrated in the scheme shown on

Methods introduced by Paul Karrer in 1927[5, 14] for the purpose of establishing the precise nature of the phenyl residue in position 2 and the points of linkage of the sugar residues have proved fruitful and reliable. Prior to Karrer's work the position of the methoxyl residues in the anthocyanidin groups VII, VIII, and IX was not known, since

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* Supplementary evidence which indicated that the anthocyanidins contain the 2-phenylbenzopyrylium nucleus was the reduction (in vitro), of the flavonol quercetin (XXIV) to cyanidin (XXV). See reference 6.

IV
Pelargonidin

XI
p-Hydroxybenzoic Acid

V
Cyanidin

XII
Protocatechuic Acid

X
Phloroglucinol

VI
Delphinidin

XIII
Gallic Acid
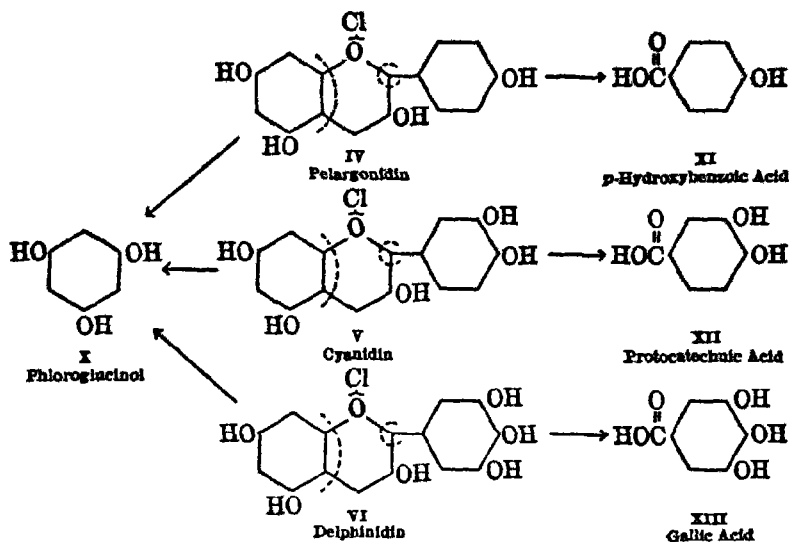
the concentrated alkali employed to degrade the pigments also removed the methoxyls. The position of the sugar residues was likewise an open question. Karrer's degradation of the sugar-free pigments with dilute barium or sodium hydroxide (10 per cent) in an atmosphere of hydrogen, which yielded the phenolic acid with the methoxyl groups intact, was, therefore, a significant advance. The results obtained by this method were confirmed through a second method wherein a degradative oxidation with hydrogen peroxide was first employed to open the ring of the anthocyanidin between carbon atoms numbers 2 and 3 without removing either the sugar residue or the methoxyl groups. The resulting intermediate, which was obtained from malvin,* could subsequently be quantitatively hydrolyzed with dilute acid or alkali to the corresponding methoxylated phenolic acid, e.g., syringic acid. The derivative of phloroglucinol, which might be formed in this hydrolysis from the various methoxylated anthocyanidins and which would contain one of the sugar residues and an acid side chain, has so far not been isolated in a crystalline condition.

The course of these degradations can be illustrated with the diglucoside, malvin chloride (XX). Starting with malvidin chloride (VIII), degradation with dilute alkali gave on the one hand phloroglucinol (X) and syringic acid (XV) or 3,5-dimethoxygallic acid. The oxidative degradation with hydrogen peroxide transformed malvin chloride (XX)

* The corresponding intermediates from other members of the group have not been isolated.

Into an intermediate malvon, whose exact constitution is not known, but which may be represented either by the structure XIVa or XIVb.

If malvon is XIVa, degradation with dilute sodium hydroxide would yield syringic acid (XV) and a derivative of phenylacetic acid (XVI), 2-glucosido-4,6-dihydroxyphenylacetic acid (not isolated). If XIVb is the correct structure for malvon, syringic acid (XV) would likewise be formed and a derivative of mandelic acid (XVII), 2-glucosido-4,6-dihydroxymandelic acid (not isolated). As the structure of syringic acid (XV) was known from previous studies in the tannin group, the positions of the two methoxyls in the 3',5'-positions of malvin are established. The structure of the two phloroglucinol residues (XVI) and (XVII) is still an open question since neither has been isolated, nor have they been prepared through independent syntheses.

VIII
Malvidin chloride

X
Phloroglucinol

XV
Syringic acid

XX
Malvin chloride
[oxidative degradation]

XIVa
Malvon?

(Isolated products)
$C_6H_{12}O_6$
(glucose)

XIVb
Malvon?

XVI
2-Glucosido-4,6-dihydroxy-phenylacetic acid
(not isolated)

XV
Syringic acid

XVII
2-Glucosido-4,6-dihydroxy-mandelic acid
(not isolated)
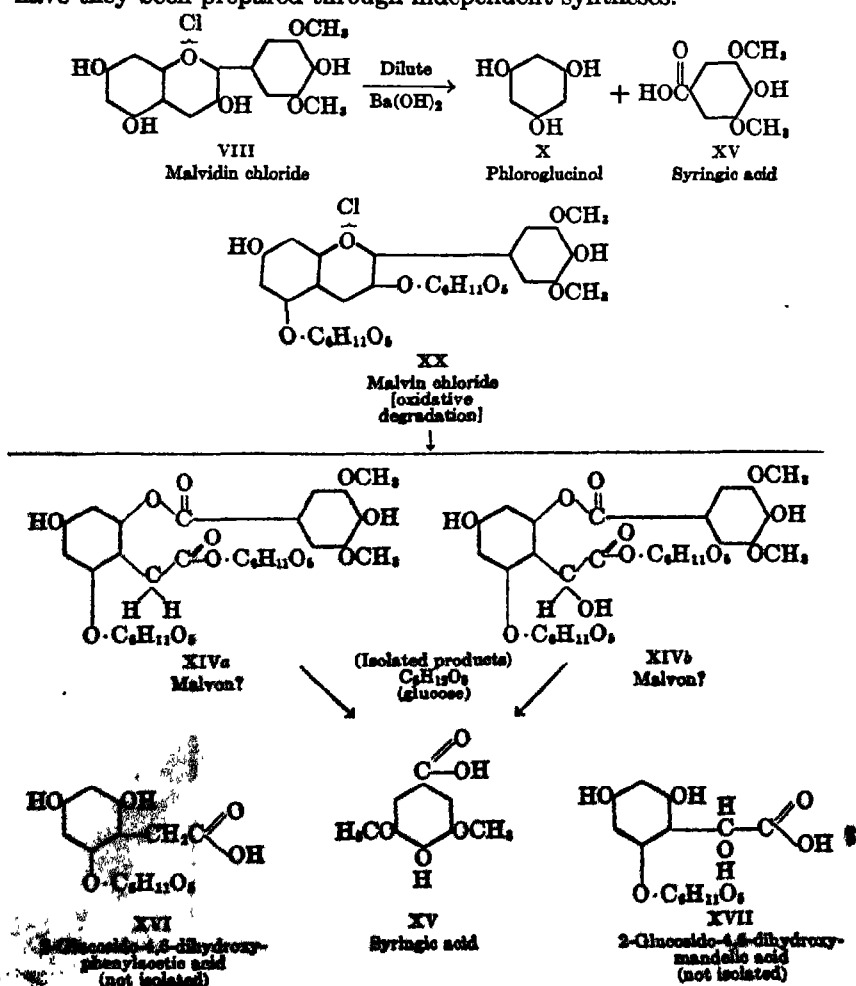
Another mode of attack introduced by Karrer[14] involved the methylation of the anthocyanins themselves, which was followed by a subsequent removal of the sugar group and the identification of the unmethylated position which it originally occupied. It was largely through these approaches that the location of the sugar residue in the monoglycosides was allocated to the 3-hydroxyl position of the anthocyanidin nucleus.

Karrer's work in conjunction with Robinson's synthetical approach, which was being made at about the same time, eventually led to the conclusion that the second sugar residue in the diglucosides occupies position 5 most generally. The total synthesis of malvin chloride (XX) realized by Robinson represents the crowning achievement of this phase of the anthocyanidin studies (see p. 1324).

General Methods of Synthesizing the Anthocyanidins and the Anthocyanins. The constitution originally assigned by Willstätter to the three parent types of the class, e.g., pelargonidin (IV), cyanidin (V), and delphinidin (VI), has been confirmed through syntheses carried on independently in the laboratories of Willstätter[6,7] and Robinson.[8,10,11,12,13] The resulting synthetic specimens have been carefully compared and identified with the natural products. The two general methods that have been employed are:

1. The addition of aryl Grignard reagents to coumarins:
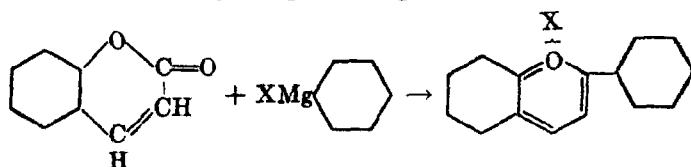
2. The condensation of o-hydroxybenzaldehydes with appropriate ketones followed by ring closure:
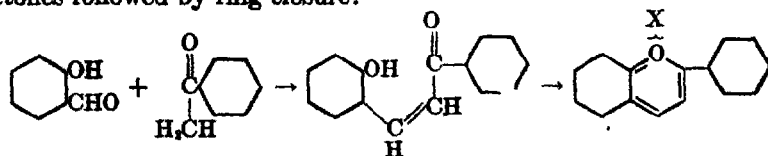
Willstätter[6] used the first of these methods in the synthesis of pelargonidin and cyanidin. Robinson[10,15] has employed the second method with eminent success in the synthesis of *all* the anthocyanidin types.

The total synthesis of several naturally occurring anthocyanins* by Robinson and his school[10,15,16] represents an even greater achievement than the synthesis of the six type anthocyanidins. The general

[14] Robinson and co-workers, *J. Chem. Soc.*, 2665, 2701 (1931); 2299 (1932).

* These are chrysanthemin, oenin, pelargonin, cyanin, and malvin.

[16] Robinson and co-workers, *ibid.*, 125, 188 (1924); 127, 166 (1925); 1068 (1926).

procedure of Robinson's method is illustrated by the synthesis of malvin, the 3,5-diglucoside of malvidin, which occurs in the wild mallow and in certain primulas.[16]

Malvidin is 3,5,7,4'-tetrahydroxy-3',5'-dimethoxy-2-phenylbenzo-pyrylium chloride (VIII). The synthesis of the diglucoside malvin was accomplished in the following manner. The 2-O-acetylglucoside of phloroglucinaldehyde (XVIII) (2-O-tetra-acetyl-β-glucosidylphloro-glucinaldehyde) was condensed with ω-O-tetra-acetyl-β-glucosidoxy-4-acetoxy-3,5-dimethoxyacetophenone (XIX) in dry ethyl acetate solution with hydrogen chloride.* The resulting condensation product was treated with barium hydroxide to remove the acetyl groups from the sugar residues. Then the flavylium salt, malvin chloride, was generated by treatment with hydrochloric acid. No divergences in the properties and behavior of the natural pigment (isolated by Karrer) and the synthetic could be detected. The important reactions involved in this synthesis are presented structurally in the scheme below:
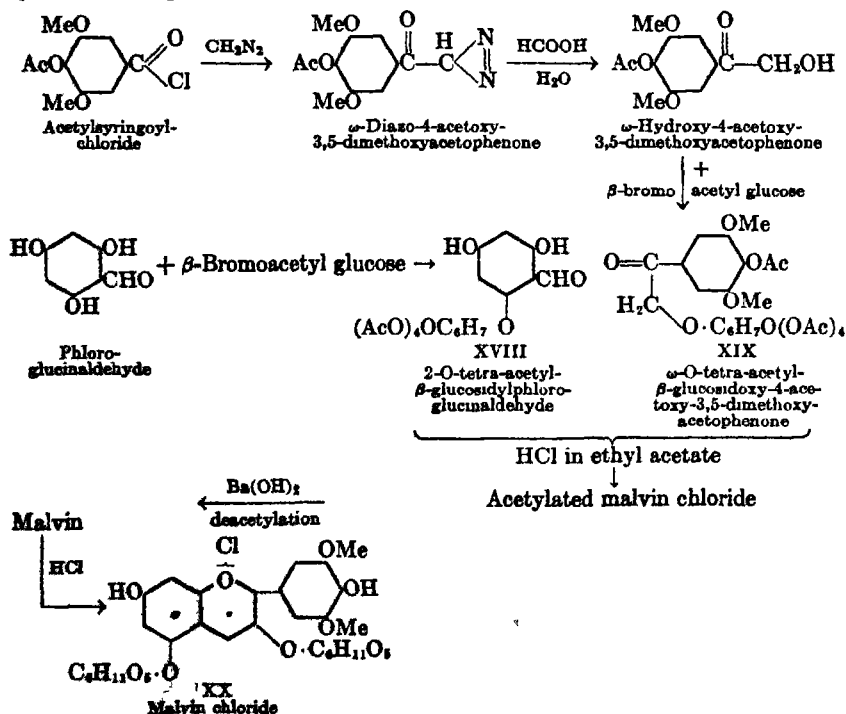
General Properties and Isolation of the Anthocyanins.[4, 5, 7, 17] As might be expected from the usual occurrence of these pigments in the

* The letter ω (omega) is used to denote a substituent at the end of any chain.
[17] Robinson and Robinson, *Biochem. J.*, **25**, 1687 (1931); **26**, 1647 (1932); **27**, 206 (1933); **32**, 1661 (1938). Price and Sturgess, *ibid.*, **33**, 1658 (1938).

plant cell sap, all members of the group are soluble in water. They are also quite soluble in the hydroxylic solvents, but they are insoluble in such non-hydroxylic solvents as ether, benzene, or chloroform. Since they cannot be extracted from the plant tissue by means of the volatile solvents, special means of separating the accompanying water-soluble substances had to be developed.

Willstätter [6, 7] recognized at the outset that these pigments are amphoteric substances and that they form true oxonium salts with acids. These salts are remarkably stable and have extraordinary crystallizing properties. Consequently, in the final stages of the isolation, the pigment is usually converted into its hydrochloric or picric acid salt.

The isolation of a member of this group usually proceeds along the following general lines. The pigment is first extracted from the plant material with methyl or ethyl alcohol containing hydrochloric acid. The crude chloride is then precipitated with ether. It is purified by redissolving in aqueous hydrochloric acid; a suitable quantity of alcohol is added and then ether to effect a reprecipitation of the salt. The final recrystallization may be done with alcoholic hydrochloric acid or aqueous alcoholic hydrochloric acid.

Karrer [18] has shown that at least some of the anthocyanins obtained by the above procedure can be purified through the use of the chromatographic adsorption technique of Tswett and that certain pigments in this group heretofore regarded as pure are mixtures containing traces of other anthocyanins.

The acid salts of the anthocyanins and anthocyanidins are usually red; the metallic salts with bases are blue. In the neutral state the pigments are purple. Thus, cyanin, the pigment of the blue cornflower and of the rose, is red in solutions of pH 3.0 or less, violet at pH 8.5, and blue at pH 11.0. The red, violet, and blue forms are the oxonium salt (XXI), the color base * (XXII), and the salt of the color base (XXIII) (after Willstätter). †

XXI
Oxonium salt of cyanin

XXII
Color base of cyanin
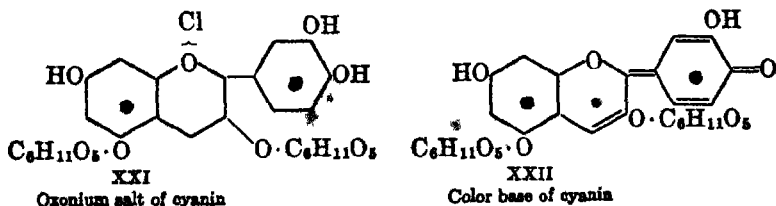
[18] Karrer and co-workers, *Helv. Chim. Acta.*, 19, 28, 1025 (1936).

* As yet no evidence exists in regard to the assumed position of the quinonoid group and the acidic hydroxyl.

† For other methods of formulating the salts and pseudo-bases through other theories see footnote * on p. 1317.

HO$\diagdown$ ... ONa

C$_6$H$_{11}$O$_6$·O

XXIII

Salt of the color base of cyanin
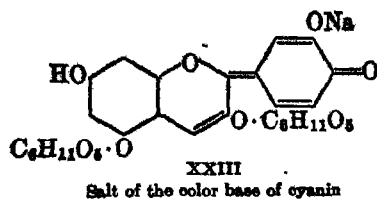
Absorption Spectra of the Anthocyanidins. The anthocyanidins as well as all the anthocyanins studied so far have strong absorption powers over the range of 6000 to 2000 Å units. The absorption spectra (p. 1783) of the sugar-free pigments and the glycosides of the pigments are approximately the same. A maximum absorption, the cause of the color, lies in the visible spectrum. All members examined also have a band that lies in the vicinity of 2700 Å. The absorption spectra of the chlorides of three anthocyanidins (concentrations 0.0001–0.00004 molar in ethyl alcohol) are presented in Fig. 1. (After Schou.[19])

λ 6000   5500   5000   4500   4000   3500   3000   2500   2000 Å

1. Pelargonidin   2. Cyanidin   3. Delphinidin
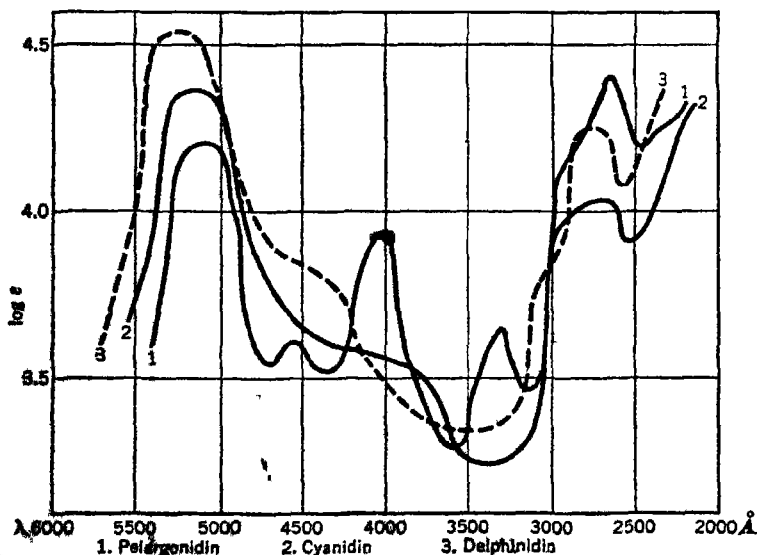
FIG. 1.

Factors Affecting the Colors of Anthocyanin Pigments in the Plant. Studies by the Robinsons [19, 17] have led to the belief that the main factors affecting the colors of the anthocyanin pigments in the cell sap are: (1) the nature and concentration of the anthocyanins and other colored substances present; (2) the state of aggregation of the antho-

cyanin in solution, which is determined in part by the pH of the cell sap and the presence or absence of protective colloids of the polysaccharide group (the pentosans); and (3) the presence or the absence of co-pigments (the tannin and flavone glycosides) and possibly also the effect of alkaloids (p. 1166), of traces of iron and other metals that form complex combinations.

The Occurrence of the Anthocyanins.[4, 5, 17] Over twenty glycosidic combinations of the various anthocyanidins have been isolated from the flowers and fruits of plants. The anthocyanins usually occur as mixtures, and the amount in the various flowers varies over a wide range. Thus, the cyanin of the blue cornflower represents 0.75 per cent of the weight of the dry petals. In certain deep red dahlias this pigment comprises over 20 per cent of the dry weight of the petals, and in the dark blue pansy the anthocyanin content (violanin) is approximately 33.0 per cent.

The Robinsons [17] have made an extensive survey on the occurrence of anthocyanins and have developed means of detecting qualitatively the type of anthocyanidin derivative that an extract of the plant tissue in question might contain. In some instances they have been able to distinguish the nature of the sugar residues (e.g., methylpentose or aldopentose) attached to the pigment.

The methods have been developed from an exhaustive study of the chemical behavior of the pure anthocyanins and anthocyanidins isolated either from natural sources or prepared synthetically. The basis for the methods are the characteristic color reactions given by the anthocyanins with alkalies and ferric chloride and the distribution coefficient of the anthocyanin between immiscible solvents. The tests employed are:

1. Oxidation test. The addition of 10 per cent aqueous sodium hydroxide to a dilute solution of the pigment which is then shaken in the presence of air. Petunidin and delphinidin are destroyed at once; the other members of the group are relatively stable.

2. Extraction with amyl alcohol; addition of sodium acetate and a trace of ferric chloride. Characteristic color reactions are observed. The color is particularly pronounced if cyanidin is present. The violet amyl alcohol solution changes to a pure blue in the last stage of the reaction. Pelargonidin, peonidin, and malvidin do not give the ferric chloride test.

3. Distribution between 1 per cent aqueous hydrochloric acid and a mixture of anisole (5 volumes) and ethyl isoamyl ether (1 volume) containing 5 g. of picric acid in 100 cc. Delphinidin is not extracted by the organic solvent layer; petunidin is taken up to some extent, cyanidin

to a considerable extent, whereas malvidin, peonidin, and pelargonidin are completely extracted if the solution is sufficiently dilute.

4. Distribution between 1 per cent hydrochloric acid and a mixture of cyclohexanol (1 volume) and toluene (5 volumes). Delphinidin and petunidin are not extracted, malvidin gives the organic solvent layer a faint blue tint, cyanidin a pale rose tint, while peonidin and especially pelargonidin are extracted to a considerable extent.

The above tests are readily applicable to crude extracts, for usually only one pigment is involved in the production of the color in the plant material extracted.

The Relationship of the Anthocyanidins to Other Classes of Plant Products.[5, 12, 13, 20, 21, 22, 23, 24] The anthocyanidins represent a class of substances which from the standpoint of degree of oxidation lie intermediate between the flavonols and the catechins. This is illustrated by a comparison of the anthocyanidin cyanidin (XXV), to the flavonol quercetin (XXIV), and to d,l-epicatechin (XXVI).

XXIV
Quercetin (flavonol)

XXV
Cyanidin (anthocyanidin)

XXVI
d,l-Epicatechin
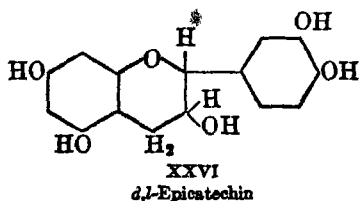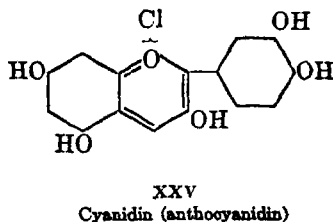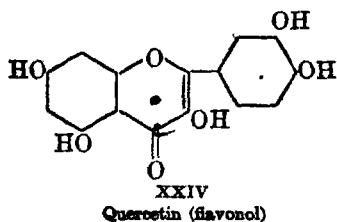
In fact, cyanidin has been obtained *in vitro* from quercetin, by means of reduction with magnesium in aqueous methyl alcoholic hydrochloric acid solution,[5] and the reduction of cyanidin to d,l-epicatechin has also been realized.[20] The reverse reaction, that is, the oxidation of the less oxidized substance to a higher stage of oxidation, has so far not been achieved.

The successful conversion of a widely distributed anthoxanthin (quercetin [XXIV]) into a widely distributed anthocyanidin (cyani-

[20] Freudenberg and co-workers, *Ann.*, **444**, 135 (1925).

[21] Whalley, *Nature*, **129**, 601 (1932).

[22] Stewart, "Recent Advances in Organic Chemistry," Longmans, Green and Co., London (1930), 6th ed., Vol. II, Chapter VII.

[23] Robinson and Robinson, *Nature*, **130**, 21 (1932).

[24] Robinson, *ibid.*, **137**, 172 (1936).

SOME OF THE IMPORTANT PROPERTIES OF THE SIX TYPE ANTHOCYANIDINS

	Pelargonidin	Cyanidin	Delphinidin	Peonidin	Malvidin or Syringidin	Hirsutidin
Color of aqueous solution	Red	Violet red	Bluish red	Violet red	Violet red	Violet red
Solubility of chloride in water	Readily soluble	Only slightly soluble	Very soluble	Readily soluble	Slightly soluble	Slightly soluble
Ferric chloride reaction	Not definite	Intense blue	Intense blue	Faint—not definite	No reaction	No reaction
Behavior toward Fehling's solution	Reduces when warmed	Reduces in the cold	Reduces in the cold	Reduces when boiled	Reduces when boiled	Reduces when boiled
Color change in soda solution	Violet then blue	Violet then blue	Violet then blue	Violet then blue	Violet then greenish blue	Violet then greenish blue
Behavior in aqueous solution	Color fades on standing	Color disappears on heating	Slow fading in the cold; when heated, rapid fading	Color disappears on heating	In very dilute solution color disappears when heated	In very dilute solution color disappears when heated

din [XXV]) has led to speculations that similar reactions occur in the plant and indicate the course of the phytochemical synthesis. Robinson [5, 12, 24, 25] is, however, of the opinion that there is little justification for this view and has suggested that the flavones and anthocyanidins are independently synthesized from a common starting point through a transformation involving an oxidation. Continuing the work of Rosenheim,[26] the Robinsons have established the presence in plant tissue of colorless precursors (called leucoanthocyanidins) which yield colored anthocyanin-like pigments on treatment with hydrochloric acid in the presence of oxygen. The leucoanthocyanidins occur as glycosides and sugar free.[25] They are classified on the basis of solubility in water and the property of being extracted from aqueous solutions with ethyl acetate.

A detailed study of the mechanism of the biosynthesis of the flavonols, anthocyanidins, and catechins will without doubt lead to many interesting results which should be of great significance to our understanding of phytochemical processes in general.*

THE DISTRIBUTION AND OCCURRENCE OF SOME REPRESENTATIVE MEMBERS OF THE SIX TYPE ANTHOCYANIDINS

1. Pelargonidin Derivatives

Pelargonin	Diglucoside	Scarlet pelargonium, orange-red dahlia, red cornflower
Punicin	Diglucoside (seemingly identical with pelargonin)	Punica granatum
Monardaein or salvianin	Diglucoside—contains also p-hydroxycinnamic acid and malonic acid	Monarda didyma and Salvia splendens, Selle, and coccinea L.
Callistephin	Monoglucoside	Callistephus chinensis, Nees, syn. Aster chinensis L.

2. Cyanin Derivatives

Cyanin	Diglucoside	Red rose, blue cornflower, deep red dahlia
Mekocyanin	Diglucoside	Dark red Mohn (Papaver Rhoeas L.)
Keracyanin	Rhamnoglucoside	Black (dark) cherries
Sambucin	Monoglucoside (apparently identical with chrysanthemin)	Elderberries (Sambucus nigra)
Idaein	Galactoside	Cranberries (mountain)
Chrysanthemin or asterin	Monoglucoside	Scarlet-red winter aster

[25] Robinson and Robinson, J. Chem. Soc., 744 (1935).
[26] Rosenheim, Biochem. J., 14, 73 (1920).

* For details on the biological significance of the anthocyanins see references 4, 22, 24, and 25. The distribution of the individual pigments among the flowering plants is treated in references 3, 4, 5, and 17. See also the general references on p. 1340.

3. *Delphinidin Derivatives*

Delphinin	Diglucoside	*Delphinium Consolida* L.
Violanin	Rhamnoglucoside	*Viola tricolor* L.
Gentianin	Monoglucoside, contains p-hydroxycinnamic acid	*Gentiana acaulis, Gentiana vulgaris*

4. *Peonidin Derivatives*

Peonin	Diglucoside	Red peony
Oxycoccicyanin	Monoglucoside	Fruit of *Oxycoccus macrocarpus* Pers.

5. *Malvidin (Syringidin) Derivatives*

Malvin	Diglucoside	Wild Malve, *Primula viscosa*
Oenin	Monoglucoside	Blue grape

6. *Hirsutidin Derivatives*

Hirsutin	Diglucoside	*Primula hirsuta*

THE FLAVONES

Introduction. The flavones (from the Latin for yellow) represent an important group of pigments that occur in the plant kingdom.[1, 2, 3, 27, 28, 29] Of all the natural pigments that can be used as dyestuffs they are by far the most widely distributed in nature.[*] They occur naturally in combination with rhamnose or glucose as glycosides, sometimes uncombined, and frequently also associated with tannins. One of the members of this group, luteolin, the main coloring matter of the herbaceous plant known as weld (*Reseda luteola*), is said to be the oldest European dyestuff known.[1, 3] Some of the flavone dyestuffs that are still significant economically are weld, young and old fustic, and quercitron bark. The use of these, however, is largely confined to the uncivilized or semi-civilized countries in which they abound. The chemistry of the flavones, which bears a striking resemblance to the anthocyanidin group (p. 1318), was elucidated largely through the researches of von Kostanecki, Herzig, and A. G. Perkin, and dates from the period of 1895 onward.

The basic unit of the flavones is γ-pyrone (I), the anhydride of an unsaturated 1,5-dihydroxy-3-ketone. γ-Pyrone, a colorless solid, has been prepared synthetically by Claisen.[30] The simplest aromatic

[27] Klein (editor), "Handbuch der Pflanzenanalyse," Springer, Vienna (1932), Vol. III, pp. 851–941.

[28] Abderhalden, "Biochemisches Handlexikon," Springer, Berlin (1911), Vol. VI.

[29] Bömer, Juckenack, and Tillmans, "Handbuch der Lebensmittel Chemie," Springer, Berlin (1933), Vol. I, p. 604.

[*] That their distribution in plants is practically universal can be readily demonstrated by the color reaction with alkalies. This reaction is best shown by colorless parts of plants, such as white flowers. Placed in ammonia vapor, almost any white flower will turn bright yellow.

[30] Claisen, *Ber.*, **24**, 118 (1891).

derivative of γ-pyrone is benzopyrone (II), commonly called chromone. Substitution of a benzene residue in position 2 of the γ-pyrone nucleus produces 2-phenylbenzopyrone (III), or flavone. When the hydrogen on carbon atom 3 in the γ-pyrone ring of flavone is substituted by hydroxyl, 3-hydroxyflavone (IV), or flavonol, is formed.
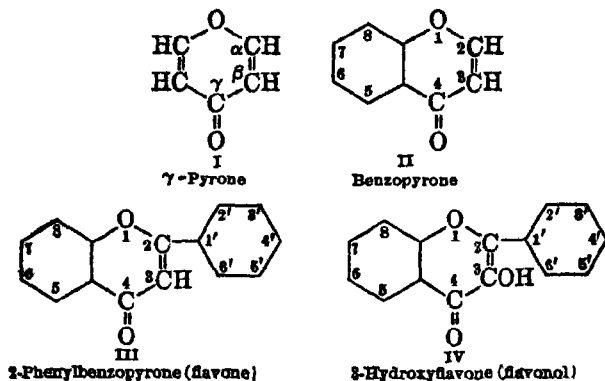
The various flavones and 3-hydroxyflavones, or flavonols, differ from III and IV, respectively, in that substitution of hydrogen atoms by hydroxyl groups has taken place in either the phenyl or benzo radical of the parent formulas. The accompanying table lists a few of the typical members of the group and illustrates the comparative constitution.* The structure of the members listed here has been verified through degradation studies and by syntheses.

Properties of the Flavones. Most of the flavones are yellow crystalline solids (with high melting points), soluble in water, alcohol, dilute mineral acids, and alkalies. From their solutions they may be precipitated by lead acetate, the precipitate being yellow, orange, or red. With ferric chloride a dull green or sometimes a red-brown coloration results. (A useful qualitative reaction for the detection of flavones is the boric acid test of Wilson.[31] The flavanones give no reaction. Some of the limitations of the test are considered by Wolfrom [32] in his studies on the isoflavones.) The solubility of the flavones in acids is due to the basic properties of the oxygen atom in the γ-pyrone nucleus. The oxygen atom by becoming tetravalent can form additive compounds with acids

* For a detailed compilation of the structure, physical properties, mode and place of occurrence of the many flavone pigments that have been studied to date see references 1, 2, 3, 27, 28, 29, 30.

[31] Wilson, J. Am. Chem. Soc., 61, 2303 (1939).

[32] Wolfrom et al., ibid., 63, 1248 (1941).

producing oxonium salts. The salts are, as a rule, more highly colored than the bases from which they are derived, and are generally very unstable in the presence of water. The flavones differ in this respect from the anthocyanidins, which yield stable oxonium salts and frequently occur as such in the plant * (pp. 1316–1317).
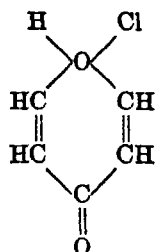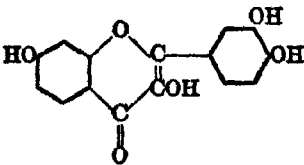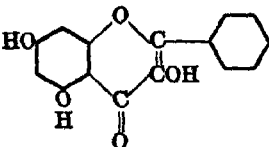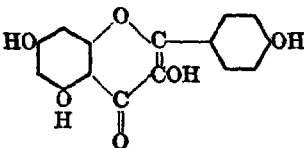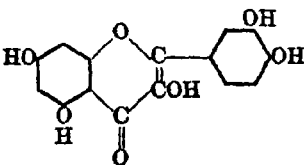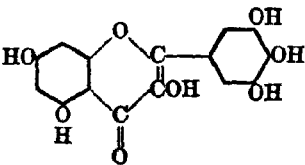
$$\gamma\text{-Pyrone}\cdot\text{HCl}$$

REPRESENTATIVE FLAVONE PIGMENTS

Name	Structural Formula	Occurrence
Flavone $C_{15}H_{10}O_2$ [2-phenylbenzopyrone]		As dust on flowers, leaves, and seeds of various primulas
Chrysin $C_{15}H_{10}O_4$ [5,7-dihydroxyflavone]		In buds of several varieties of poplar (*P. nigra, P. pyramidalis*)
Apigenin $C_{15}H_{10}O_5$ [5,7,4'-trihydroxyflavone]		In parsley as glycoside apiin; in yellow dahlias
Luteolin $C_{15}H_{10}O_6$ [5,7,3',4'-tetrahydroxyflavone]		In weld (*Reseda luteola*), dyers' broom (*Genista tinctoria*)

* For details on the biological significance of the flavones, see reference 4; also Haas and Hill, "An Introduction to the Chemistry of Plant Products," 4th ed., Longmans Green and Co., London (1928), Vol. I.

REPRESENTATIVE FLAVONE PIGMENTS—*Continued*

Name	Structural Formula	Occurrence
Fisetin $C_{15}H_{10}O_6$ [3,7,3',4'-tetra-hydroxyflavone]		In wood of young fustic (*Rhus cotinus* and *Quebracho colorado*)
Galangin $C_{15}H_{10}O_5$ (flavonol of Chrysin) [3,5,7-tri hydroxyflavone]		In galanga root, the rhizome of *Alpina officinarum*
Kaempferol $C_{15}H_{10}O_6$ (flavonol of Apigenin) [3,5,7,4'-tetra-hydroxyflavone]		In blue delphinium flowers
Quercetin $C_{15}H_{10}O_7$ (flavonol of Luteolin) [3,5,7,3',4'-penta-hydroxyflavone]		As 3-rhamnoside in bark of American oak (*Quercus tinctoria*), leaves of horse chestnut, colored onion scales, etc.
Myricetin $C_{15}H_{10}O_8$ [3,5,7,3',4',5'-hexahydroxy-flavone]		As glycoside in an evergreen native to the Orient, the Myricaceae family

Degradation of the Flavones. On boiling with alkali the heterocyclic ring system is opened. The course of the degradation can be illustrated with flavone, which forms *o*-hydroxydibenzoylmethane (V). This then degrades in part to salicylic acid (VI) and acetophenone (VII), and in part to *o*-hydroxyacetophenone (VIII) and benzoic acid (IX). On fusion with caustic alkali the flavones are degraded to a phenol and an acid. Phloroglucinol and protocatechuic acid are commonly formed, and sometimes resorcinol and resorcylic or hydroxybenzoic acids, depending on the substitution in the benzene rings in positions 2 and 5,6 of the γ-pyrone nucleus (I). Thus, quercetin and luteolin yield phloroglucinol

and protocatechuic acid (3,4-dihydroxybenzoic acid). Apigenin and kaempferol yield phloroglucinol and p-hydroxybenzoic acid.

III
Flavone

V

VI

VII

VIII

IX
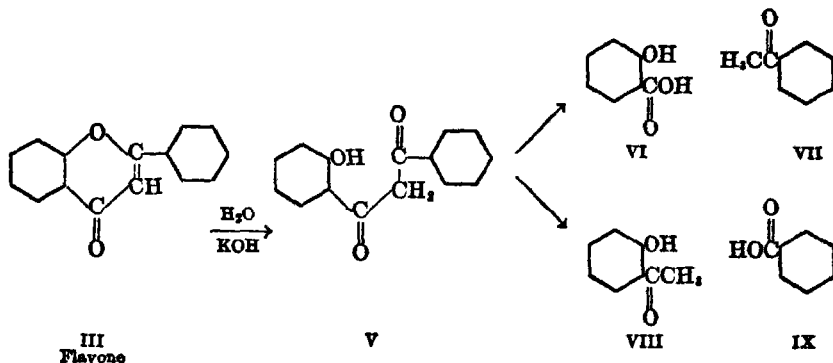
Synthesis of the flavones. Various methods have been developed by von Kostanecki, Perkin, Robinson, and others.[3, 2, 33, 34] One of the most useful general methods involves a condensation of appropriate alkylated o-hydroxyacetophenones (X) with esters of aromatic acids (XI), or esters of alkylated salicylic acid (XII) with acetophenones (XIII) in the presence of sodium.

X

XI

—ROH

XII

XIII

o-Methoxydibenzoylme-
thane [methyl ether of
formula V]

III
Flavone
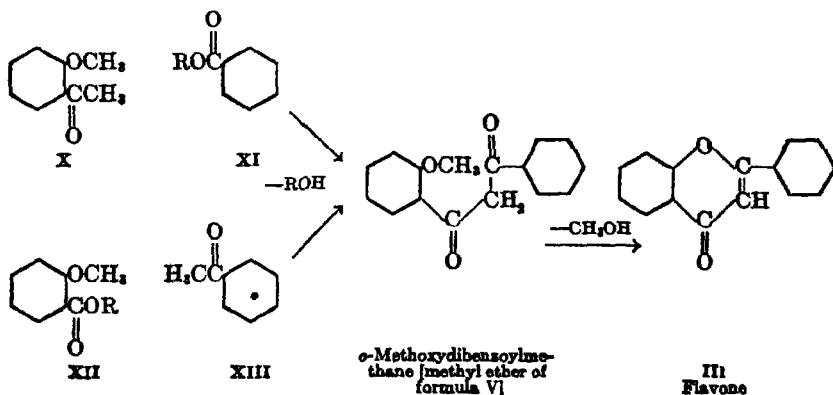
Another general method employs a condensation of o-hydroxyaceto-phenone (or its methoxyl derivative) with benzaldehyde (its hydroxyl or methoxyl derivatives). The appropriate hydroxyl or methoxyl derivatives of the two starting products are chosen depending on the flavone sought.

[33] Hepworth, "Chemical Synthesis," Blackie and Son, London (1924).

[34] Abderhalden, "Handbuch der biologischen Arbeitsmethoden," Urban and Schwarzenberg, Berlin (1922), Vol. I, Part 10, Supplement 84.

XIV
o-Hydroxyacetophenone
     +
XV
Benzaldehyde
   →
XVI
o-Hydroxybenzalacetophenone
(o-hydroxychalkone)

HCl
(alcoholic)

III
Flavone
  ←
Bromination
followed by
elimination of
HBr with NaOH

XVII
Flavanone *
[2,3-dihydroflavone or 2-phenyl-
2,3-dihydrobenzopyrone]
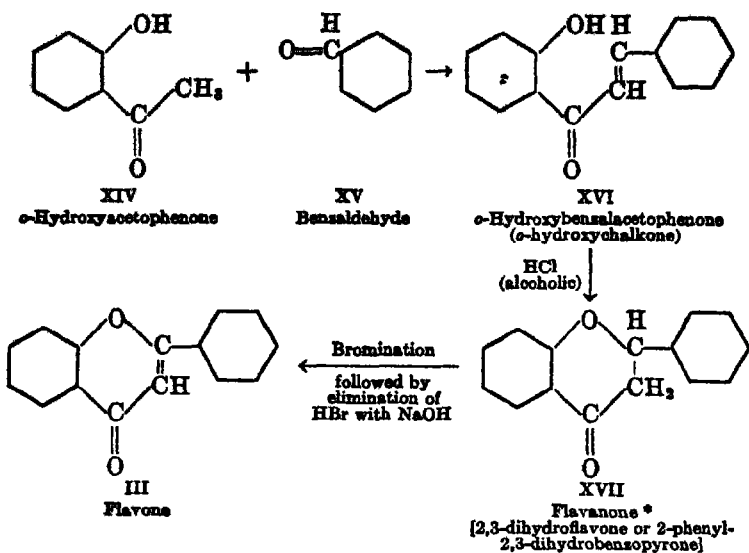
When flavanone † (XVII) is treated with amyl nitrite and strong hydrochloric acid in alcoholic solution, the oximino (isonitroso) compound (XVIII) is formed, which on boiling in acetic acid solution with 10 per cent sulfuric acid forms 3-hydroxyflavone or flavonol (XIX).

XVII
Flavanone

$C_5H_{11}ONO$
+HCl
→
XVIII
Oximinoflavanone

H_2SO_4
→
XIX
Flavonol [3-hydroxyflavone]
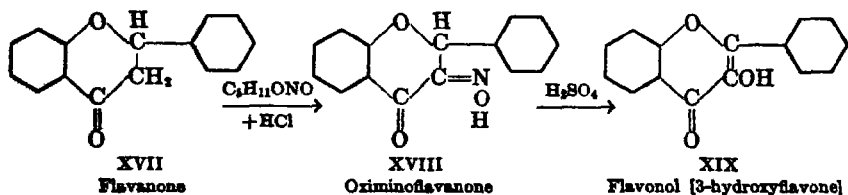
Finally, flavonol derivatives can be obtained by reacting the appropriate ω-methoxyacetophenone with appropriate phenolic acid anhydrides. An example of this reaction which was developed by Robinson is presented below.[35]

The synthesis of quercetin (XXVI) (3,5,7,3',4'-pentahydroxyflavone) was realized by von Kostanecki[36] in 1904. Phloroacetophenone-4,6-di-

* When the carbon 3 in the flavone is completely reduced with the elimination of the double bond the structure is called a *flavanone*.

† Derivatives of flavanone also occur in nature. Hesperidin, the glycoside occurring in oranges, is a flavanone. It is 5,7,3'-trihydroxy-4'-methoxyflavanone-7-rhamnoside See references 1, 2, 3, 27, 28, 29.

[35] Allan and Robinson, *J. Chem. Soc.*, **125**, 2192 (1924); **2334** (1926).
[36] von Kostanecki, Lampe, and Tambor, *Ber.*, **37**, 1402 (1904).

methyl ether (XX) was condensed with veratric aldehyde (XXI). The resulting 2-hydroxy-4,6,3',4'-tetramethoxychalkone (XXII) was then boiled with dilute sulfuric acid in methyl alcohol and converted into the 2,3-dihydrotetramethoxyflavone (XXIII). The introduction of the hydroxyl on carbon 3 in the pyrone ring was done in the usual manner through the oximino compound (XXIV). Finally, removal of the four methoxyl residues from tetramethoxyquercetin (XXV) produced quercetin (XXVI), identical in all respects with the naturally occurring pigment. The various stages of this synthesis can be illustrated structurally as follows:
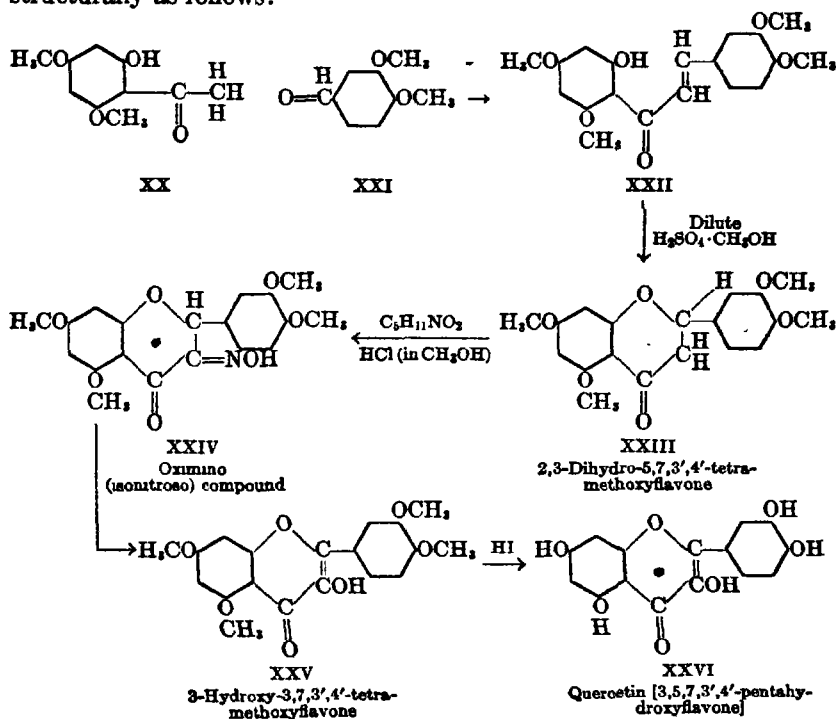
A direct synthesis of quercetin (XXVI) was realized in 1926 by Robinson.[38] ω-Methoxyphloroacetophenone (XXVII) was condensed with the anhydride of 3,4-dimethoxybenzoic acid (veratric anhydride) (XXVIII) in the presence of the potassium salt of veratric acid. A molecule of veratric acid is regenerated as a result of the condensation which produces the 3,3',4'-trimethyl ether of quercetin (XXIX). Removal of the methoxyl residues from the trimethoxyquercetin (XXIX) yielded the free pigment (XXVI), m.p. 313–314°, identical in all respects with the naturally occurring product.

$$XXVII \xrightarrow{K\text{-veratrate}} XXIX \rightarrow XXVI$$

XXVII
ω-Methoxyphloro-
acetophenone

XXVIII
Veratric anhydride

XXIX
3,3',4'-Trimethyl ether
of quercetin

XXVI
Quercetin
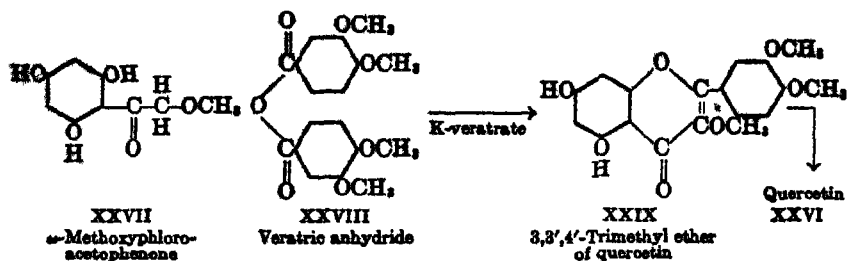
ISOFLAVONES

The isoflavones have the phenyl group in position 3 of the benzo-pyrone nucleus.

XXX
Isoflavone
3-Phenylbenzopyrone

XXXI
Daidzein
7,4'-Dihydroxyisoflavone

XXXIII

XXIV

XXXII
2,4-Dihydroxyphenyl-4'-hydroxybenzylketone

Dilute
alkali
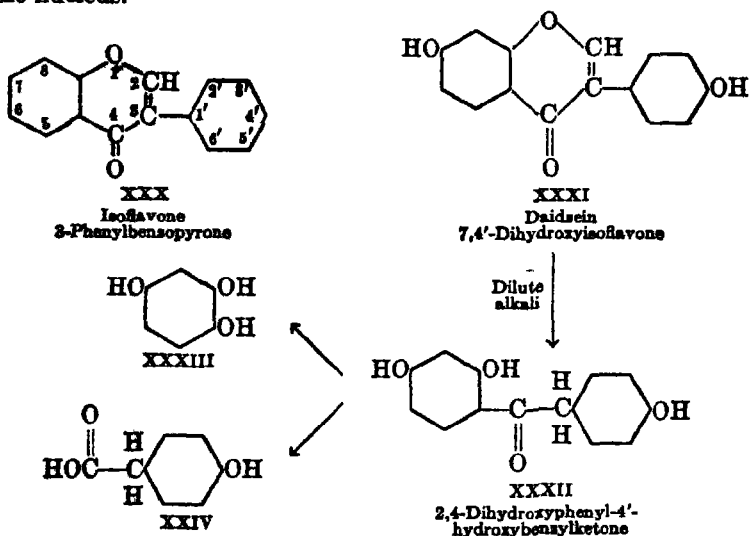
A characteristic of the isoflavones (XXX) is that treatment with mild alkali produces, quantitatively, one mole of formic acid and an hydroxylated benzyl-o-hydroxyphenylketone, which on further treatment with strong alkali degrades to an hydroxylated phenylacetic acid and a polyhydric phenol. Thus daidzein (XXXI) yields first the intermediate ketone (XXXII) and formic acid and finally 1,2,4-trihydroxybenzene (XXXIII) and p-hydroxyphenylacetic acid (XXXIV).[57]

The synthesis of isoflavone (XXX) involves the interaction of

[57] Wals, Ann., 489, 118 (1931).

o-hydroxyphenyl benzyl ketone (XXXV) with ethyl formate in the presence of sodium dust.

XXXV

$\left[\begin{array}{c}\text{Intermediate}\\ \text{not isolated}\end{array}\right]$

$\downarrow -H_2O$

XXX
Isoflavone
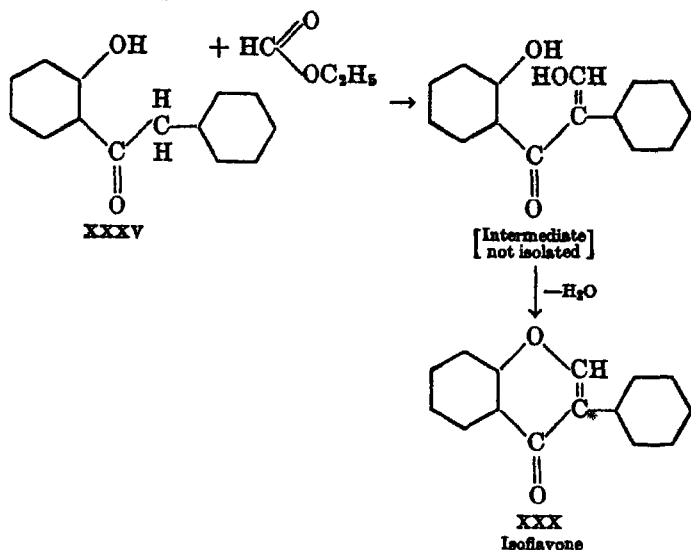
The synthesis of substituted isoflavones follows along similar lines from the appropriately substituted ketone. Thus daidzein, $C_{15}H_{10}O_4$ (XXXI), the aglycone of the monoglucoside daidzin, $C_{21}H_{20}O_9$, from the bean *Soja hispida*, is prepared by reacting 2,4-dihydroxyphenyl-4'-hydroxybenzyl ketone (XXXII) with ethyl formate in a sealed tube.[38]

XXXII

Daidzein (XXXI)
7,4'-Dihydroxyisoflavone
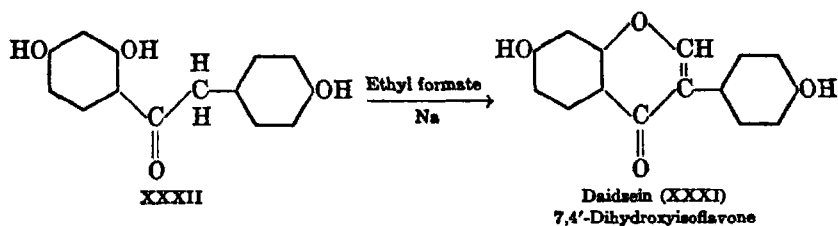
For recent studies on the isoflavone group, the papers by Wolfrom and co-workers on the pigments of the osage orange are recommended.[39]

[38] Wessely, Kornfeld, and Lechner, *Ber.*, **66**, 685 (1933).
[39] Wolfrom, *J. Am. Chem. Soc.*, **63**, 1248, 1253 (1941).

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Summary reports on current progress in the field appear in *Annual Review of Biochemistry*, Stanford Press, Stanford, Vol. I (1932); Vol. V (1936); Vol. IX (1940). See, also, *Annual Reports of the Chemical Society (London)*, Vol. I (1904), to Vol. XXXVII (1940).

For excellent summaries of the work on anthocyanins, the reader's attention is called to the reviews by Professor Robinson and his associates:

ROBINSON, *Nature*, **137**, 94 (1936); *Ber.*, **67A**, 85 (1934).

ROBINSON, *Nature* (Royal Jubilee Number), **135**, 732–736 (1935).

ROBINSON, *Naturwissenschaften*, **20**, 612 (1932).

ROBINSON, *J. Soc. Chem. Ind.*, **52**, 737 (1933).

ROBINSON, *Nature*, **132**, 625 (1933).

ROBINSON and ROBINSON, *ibid.*, **130**, 21 (1932).

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SCOTT-MONCRIEFF, *Ergeb. Enzymforsch.*, **8**, 277 (1939).

LAWRENCE, PRICE, ROBINSON, and ROBINSON, *Trans. Roy. Soc. (London)*, **B230**, 149 (1939).

LAWRENCE and PRICE, *Biol. Rev. Cambridge Phil. Soc.*, **15**, 35 (1940).

CHAPTER 19

THE STEROIDS *

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CONTENTS

* The literature has been systematically reviewed to January, 1941, but important publications up to August, 1942, have been included. For monographs and reviews on the steroids, see end of chapter.

INTRODUCTION

Among the substances found in nature are compounds derived from the hydrocarbon cyclopentanoperhydrophenanthrene, which for simplicity are called the steroids.[1] These natural compounds are oxygenated, alkyl-substituted derivatives of the parent hydrocarbon, and have the ring system given in structure I. The recognized members of this group are the sterols, the bile acids, the cardiac aglucons, the genins of the toad poisons, the digitalis sapogenins, the sex hormones, certain adrenal substances, and a few other compounds which are not easily classified.
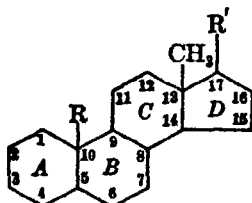
I. Ring system of the steroid group.

Variations within the group include changes (1) in the nature of the side chains R and R'; (2) in the spatial configuration of the nucleus and its substituent groups; (3) in the number and position of the hydroxyl groups; and (4) in the degree and position of unsaturation. The nature of the side chain R' changes markedly; and R, when present, represents either a methyl group, an oxidation product thereof, or hydrogen. Since the nucleus is alicyclic, stereoisomeric modifications (p. 328) of the type exhibited by the decalins occur. Actually the mutations in nuclear spatial configuration are few, since the relationship of rings B/C and C/D appears to be the stable *trans* arrangement in nearly all the known compounds. The relationship of rings A/B is not always the same, and,

<div align="center">GENERAL VARIATIONS OF TYPE FORMULA I FOUND IN NATURE</div>

Member	R	R'
Sterols	—CH$_3$	$\overset{\displaystyle CH_3}{\overset{\displaystyle |}{—CH}}$—(CH$_2$)$_3$—CH(CH$_3$)$_2$, or substituted isoöctyl
Bile acids	—CH$_3$	$\overset{\displaystyle CH_3}{\overset{\displaystyle |}{—CH}}$(CH$_2$)$_2$—COOH
Heart principles	—CH$_3$, —CH$_2$OH —CHO	Four- or five-carbon unsaturated lactone ring
Digitalis saponins	—CH$_3$	Eight-carbon ketospiroacetal (?)
Sex hormones		
Estrogenic*		Hydroxyl or carbonyl group
Corpus luteum	—CH$_3$	Acetyl group
Androgenic	—CH$_3$	Hydroxyl or carbonyl group
Adrenal substances	—CH$_3$	Hydroxyethyl, α,β-dihydroxyethyl or hydroxyacetyl (with H or OH at C$_{17}$).

<div align="center">* Ring A, or rings A and B, are benzenoid.</div>

through usage, those compounds which have the *trans* configuration are designated as *allo* structures. Although R and the C$_{13}$—CH$_3$ group may be attached so that they project either above or below the plane of the paper, they are usually assumed to be projecting above the plane of the paper. Since these groups are generally methyl radicals and are attached at an angle to a carbon atom shared by two rings, they are referred to as angular methyl groups.

Most of the steroids are hydroxylated at C$_3$, and many of them are hydroxylated elsewhere in the ring system or the side chains. These hydroxyl groups may be attached either *cis* or *trans* to any given reference point. The difficulty of establishing such relationships unequivocally and of agreeing upon a standard reference position has led to a fairly general adoption of the designations α and β to describe the two isomers of an epimeric pair. If the assumption is made that the angular methyl groups at C$_{10}$ and C$_{13}$ project forward, then the α-configuration is probably in a *trans* position, while the β-configuration is in a *cis* position to these two reference points. In the structural formulas the α-configuration is indicated by dotted lines and the β-configuration by solid lines. Later in this discussion methods of determining the stereochemical relationships will be considered.

Generalizations as to the position and degree of unsaturation cannot be made. The members of the group exhibit varying degrees of unsatura-

tion, but in no instance, among the natural compounds, is the entire ring system aromatic.

The chemical study of any group of natural compounds passes through three stages: isolation, constitutional determination by degradation, and synthesis. Among the steroids the more complicated members, the sterols, the bile acids, and the cardiac glycosides, were the first to be isolated and studied. The simpler sex hormones were not known until 1929, and one of these is the only natural steroid that has been completely synthesized. Serious handicaps often had to be overcome in the course of isolation for, where the substances were abundantly present in nature, mixtures of closely allied compounds were usually encountered. Because of the size of the molecules, the usual methods of characterization did not always lead to satisfactory conclusions, and, in many instances, new physical and biochemical methods were necessary for precise definition. The sex hormones and the adrenal substances presented further problems, since at first only microscopic amounts were available for study. Because of these difficulties, microchemical methods have played a large part in the development of the chemistry of this group.

Up to the year 1932 the structural investigations were concerned primarily with the nature of the nucleus. The study of this problem was carried out with the readily available cholesterol (II); with the common bile acids—cholic (III), desoxycholic (p. 1363), and α-hyodesoxycholic (p. 1426); and to some extent with the less common acids—lithocholic (p. 1361) and chenodesoxycholic (p. 1415). The early investigation of cholesterol, $C_{27}H_{46}O$, characterized this as a monohydric secondary alcohol containing one double bond and an isoöctyl side chain. Similarly, the bile acids were recognized as hydroxy derivatives of cholanic acid, $C_{24}H_{40}O_2$, which, in turn, could be shown to contain the same nucleus

$$CH_3$$
$$|$$

as cholesterol and a side chain, $-CH-CH_2-CH_2-CO_2H$. After allowing for the demands of the side chains, it was evident that the nucleus was hydroaromatic in nature, and apparently made up of four condensed rings. Owing to the lack of hetero atoms, the nature of the nucleus had to be determined by the methods of oxidative degradation and of dehydrogenation.

In 1928 Windaus[2] and Wieland[3] reviewed in Nobel Prize addresses the results of their investigations on the sterols and the bile acids. The structures of cholesterol (IIa) and cholic acid (IIIa) which they dis-

[2] Windaus, "Le Prix Nobel," Stockholm (1928).
[3] Wieland, "Le Prix Nobel," Stockholm (1928).
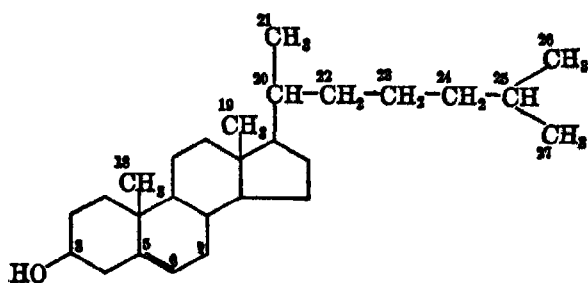
II. Cholesterol
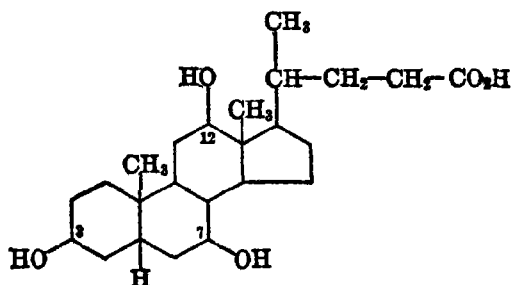
III. Cholic acid
(3,7,12-Trihydroxycholanic acid)
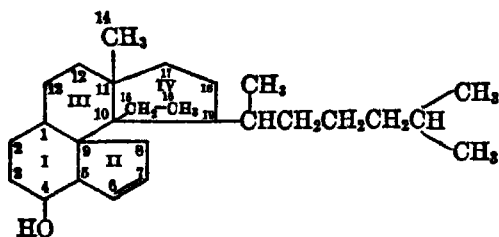
IIa. Old cholesterol structure
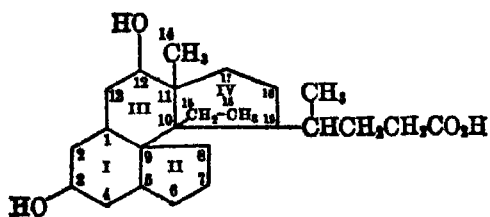
IIIa. Old cholic acid structure

cussed had been evolved by a study of the products of oxidative degradation and seemed established in all details save for the attachment of carbon atoms 15 and 16. These were assumed to be present as an ethyl group at C_{10}. Subsequent attempts by Wieland[4] to prove the position of the ethyl group led to the startling conclusion that there was no such grouping attached to ring IV of structures IIa and IIIa at the point in question. The two carbon atoms accordingly became "obdachlos" (homeless), and later investigation was concerned largely with attempts to place them in the ring nucleus. Wieland[5] and Borsche[6] both suggested structures in which the group CH_3—$CH<$ was inserted in ring III, but the resulting seven-membered ring structures were never entirely acceptable.

A few years later, in 1932, Rosenheim and King[7] called attention to a neglected piece of evidence—the formation of chrysene as a product of selenium dehydrogenation of cholesterol and cholic acid. On the basis of this fact, and of the x-ray measurements of ergosterol (p. 1399) and of calciferol (p. 1409) by Bernal,[8] it was suggested that the ring nucleus of the sterols and of the bile acids was perhydrochrysene. Study of the evidence in the light of this suggestion led Rosenheim and King[9] and Wieland and Dane[10] to modify the perhydrochrysene to a cyclopentanoperhydrophenanthrene nucleus. This new structure for the sterols and bile acids was immediately compatible with the vast amount of experimental material which had been accumulated.[*]

With the investigations of the sterols and of the bile acids as a background, the structural examination of most of the other members of the group was conducted to a satisfactory conclusion with rapidity. Although in many instances only small amounts of these natural products were available for study, degradation to mutually common compounds was carried out in nearly all cases. Finally, in 1939–1940, Bachmann and co-workers carried out a total synthesis (p. 1475) of one of the sex

[4] Wieland and Vocke, Z. physiol. Chem., **191**, 69 (1930).

[5] Wieland and Deulofeu, ibid., **198**, 127 (1931).

[6] Borsche and Todd, ibid., **197**, 173 (1931).

[7] Rosenheim and King, J. Soc. Chem. Ind., **51**, 464 (1932).

[8] Bernal, Nature, **129**, 277 (1932); J. Soc. Chem. Ind., **51**, 466 (1932); for summary see Bernal et al., Trans. Roy. Soc. (London), **239A**, 135 (1940).

[9] Rosenheim and King, Nature, **130**, 315 (1932); J. Soc. Chem. Ind., **51**, 954 (1932); **62**, 299 (1933).

[10] Wieland and Dane, Z. physiol. Chem., **210**, 268 (1932).

[*] Reviews reconciling the older work with the new structure: Windaus, Z. physiol. Chem., **213**, 147 (1932); Heilbron, Simpson, and Spring, J. Chem. Soc., 626 (1933); Rosenheim and King, Ann. Rev. Biochem., **3**, 87 (1934). Reviews giving the developments: Ann. Repts. Chem. Soc. (London), **24**, 128 (1927); **25**, 157 (1928); **28**, 139 (1931); **30**, 198 (1933).

hormones. Thus, both by degradation and by synthesis, the structure of the nucleus has been established.

THE STRUCTURE OF THE NUCLEUS

As was suggested above, the structural investigation of the nucleus has been carried out exclusively with the sterols and the bile acids. The early investigators felt that the nucleus was identical in these two series, but proof was first offered by Windaus and confirmed by Wieland. With the establishment of this fact, evidence obtained from the study of cholesterol could be applied to the bile acids, and vice versa. Then followed a period of intensive work in which the several rings were opened and the products studied by thermal decomposition. This path led to a false solution, however, and it was selenium dehydrogenation· which finally furnished the essential clue.

When selenium dehydrogenation of cholesterol is carried out at 360°, one of the products is a hydrocarbon, $C_{18}H_{16}$ (IV, Diels' hydro-
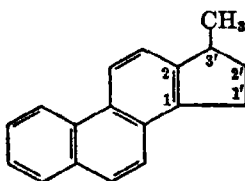
IV. 3'-Methyl-1,2-
cyclopentenophenanthrene
(Diels' hydrocarbon)

carbon). Early in 1934 the structure of this hydrocarbon was definitely established as 3'-methyl-1,2-cyclopentenophenanthrene (or γ-methyl-cyclopentenophenanthrene), but because of the drastic conditions of selenium dehydrogenation, together with the very poor yield of product, the formation of a cyclopentenophenanthrene is not good proof of a cyclopentanoperhydrophenanthrene nucleus. Although selenium dehy-drogenation of a hydrocarbon derived from certain of the bile acids to methylcholanthrene (p. 1354) in relatively good yield furnishes con-firmatory evidence of the structure of the nucleus, the real proof comes from a reinterpretation and further investigation of the oxidative degra-dation of cholesterol and of the bile acids.

Evidence of a Common Nucleus in the Sterols and Bile Acids. When cholesterol is catalytically hydrogenated at room temperature, dihydro-cholesterol is formed; at 200°, and with nickel as a catalyst, the product is a mixture of dihydrocholesterol and two of its stereoisomers, *epidi*-

hydrocholesterol and *epi*coprosterol (and traces of coprosterol).[11] After the mixture has been resolved into its components, *epi*coprosterol may be converted to coprosterol. Reduction of either of these through the stage of the chloride leads to the hydrocarbon coprostane (p. 1367). Similarly, from dihydrocholesterol and *epi*dihydrocholesterol, cholestane (p. 1367) is obtained. Oxidation of coprostane and of its stereoisomer cholestane with hot chromic acid gives, among other products, cholanic acid and *allo*cholanic acid, respectively.[12] These two acids, the parent substances of the bile acids, can be obtained by appropriate treatment of cholic or α-hyodesoxycholic acid.

Conversely, cholanic acid may be converted, through the action of isopropylmagnesium bromide on its ester, to a ketone which on Clemmensen reduction gives coprostane.[13] The proof of the identity of the nuclei of the two series may be summarized as shown:

Hyodesoxycholic acid
$C_{24}H_{40}O_2$
↓

Dihydrocholesterol   →(Reduction)   Cholestane   →(CrO₃ Oxidation)   *Allo*cholanic acid
$C_{27}H_{48}O$ $C_{27}H_{48}$ $C_{24}H_{40}O_2$
↑

Cholesterol
$C_{27}H_{46}O$
↓

*Epi*coprosterol
$C_{27}H_{48}O$
↓

Coprosterol   →(Reduction)   Coprostane   →(CrO₃ Oxidation) / ←(IsopropylMgBr and reduction)   Cholanic acid
$C_{27}H_{48}O$ $C_{27}H_{48}$ $C_{24}H_{40}O_2$
                                                                                                  ↑ (Catalytic reduction)

Cholic acid   →(Vac. dist. −3H₂O)   Cholatrienic acid
$C_{24}H_{40}O_5$ $C_{24}H_{34}O_2$

Dehydrogenation Products. The initial experiments in which cholesterol and cholic acid were dehydrogenated were conducted by Diels.[14] With palladized charcoal at *ca.* 500°, the identifiable product from cholesterol was chrysene, $C_{18}H_{12}$. With cholesteryl chloride at 340–360° and with selenium in place of palladized charcoal, two hydrocarbons, $C_{18}H_{16}$ and $C_{25}H_{24}$, were obtained. The latter gave reactions suggestive of a fluorene nucleus and was thought at the time to indicate

[11] Windaus, *Ber.*, **48**, 1724 (1915). See p. 1374 for formulas.
[12] Windaus and Neukirchen, *Ber.*, **52**, 1915 (1919).
[13] Wieland and Jacobi, *Ber.*, **59**, 2064 (1926).
[14] Diels and Gädke, *Ber.*, **60**, 140 (1927); Diels, Gädke, and Körding, *Ann.*, **459**, 1 (1927); Diels and Karstens, *Ann.*, **478**, 129 (1930).

that ring IV of the old cholesterol formula (IIa) was five-membered. After Rosenheim and King had pointed out the significance of chrysene, interest in the dehydrogenation products revived, centering especially on the compound $C_{18}H_{16}$. Since different workers could not agree on the nature of this hydrocarbon, or even on the production of chrysene, a polemical situation developed which has led to a reasonably accurate knowledge of what happens under the drastic conditions of selenium dehydrogenation.[15]

Diels [15b] and Ruzicka [15f] in particular have examined the nature of the products obtained by dehydrogenation. Cholesterol at 300–360° gives Diels' hydrocarbon and another hydrocarbon, $C_{25}H_{24}$, which, though not identical with the hydrocarbon of structure V, seems to be closely allied with it.[15a], *

V
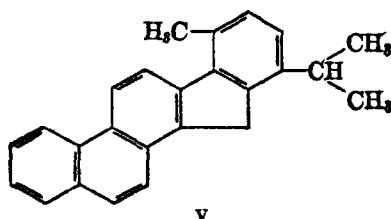
Cholic acid (or its dehydration product, cholatrienic acid), when subjected to selenium dehydrogenation, gives a variety of products, depending on the temperature at which the dehydrogenation is conducted. At lower temperatures (360°), Diels' hydrocarbon and a second hydrocarbon, which was first reported to be $C_{21}H_{16}$, are formed. Cook[16] suggested that the composition of this second hydrocarbon might be

[15] (a) Cook and Hewett, J. Soc. Chem. Ind., **52**, 451 (1933); Cook, Hewett, Mayneord, and Roe, J. Chem. Soc., 1727 (1934). (b) Diels, Ber., **66**, 487, 1122 (1933); Diels and Klare, Ber., **67**, 113 (1934); Diels and Stephan, Ann., **527**, 279 (1937). (c) Gamble, Kon, and Saunders, J. Chem. Soc., 644 (1935). (d) Raudnitz, Petrů, and Stadler, Ber., **66**, 879 (1933). (e) Rosenheim and King, J. Soc. Chem. Ind., **52**, 299 (1933). (f) Ruzicka, Goldberg, and Thomann, Helv. Chim. Acta, **16**, 812 (1933); Ruzicka, Thomann, Brandenberger, Furter, and Goldberg, ibid., **17**, 200 (1934); Ruzicka and Goldberg, ibid., **18**, 434 (1935); **20**, 1245 (1937). (g) Schlenk, Bergmann, and Bergmann, J. Soc. Chem. Ind., **52**, 209 (1933).

* With the congeners of cholesterol, such as ergosterol and sitosterol, there is a difference of opinion about the formation of $C_{18}H_{14}$. Ruzicka finds that hydrocarbons of a carbon content greater than C_{21} are produced from these sterols, but Diels has been able to obtain only $C_{21}H_{14}$, although he admits that small amounts of other hydrocarbons may be formed. Differences in temperature may be the explanation of the discrepancies in the results of the two workers. The solution of the problem of the products of dehydrogenation is rendered difficult by the fact that the yield of each hydrocarbon is less than 1 per cent.

[16] Cook, Danei, Hewett, Iball, Mayneord, and Roe, J. Chem. Soc., 1319 (1935); Bachmann, Cook, Hewett, and Iball, ibid., 54 (1936).

$C_{22}H_{16}$, rather than $C_{21}H_{16}$, and has proved his point by the synthesis of 5-methyl-2',1'-naphtho-1,2-fluorene (VI), which agrees well with the product obtained by Ruzicka. At temperatures of 400° or higher, chrysene (VII) and picene (VIII) are formed. Ruzicka has suggested the following mechanism for their formation: chrysene results from the union of the angular methyl group at C_{18} with an opened ring D; picene from an analogous type of ring enlargement with simultaneous ring formation involving the side chain.

The structure of Diels' hydrocarbon as 3'-methyl-1,2-cyclopenteno-phenanthrene, $C_{18}H_{16}$ (IV), has been definitely established through two syntheses. By the first of these,[17] 2-acetylphenanthrene is con-

III. Cholic acid

Diels' hydrocarbon
(IV)

VI, 5-Methyl
2',1'-naphtho-1,2-fluorene

VII. Chrysene

VIII. Picene
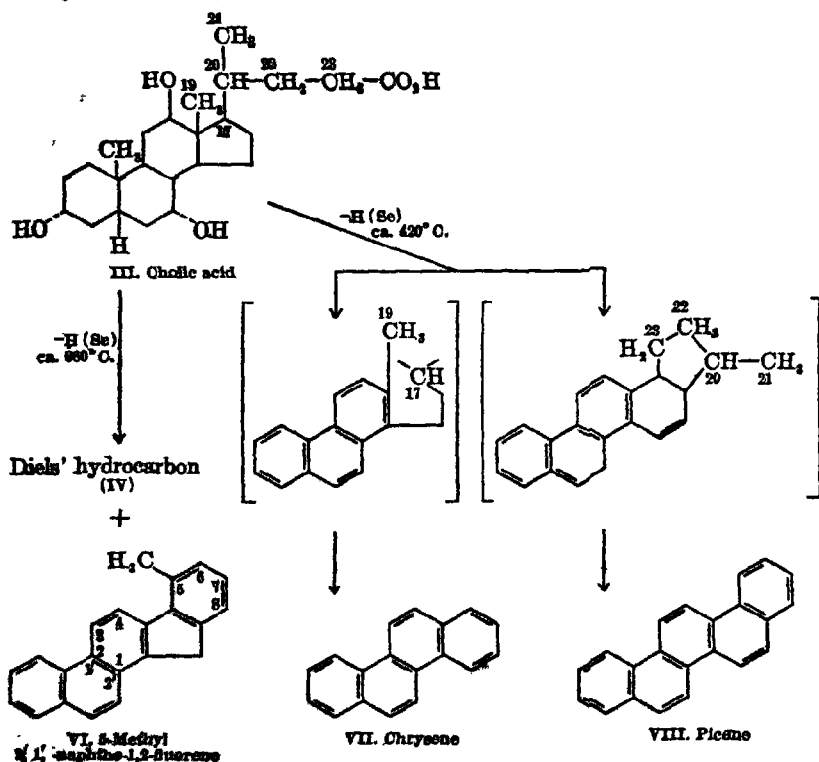
densed with bromoacetic ester, the product (IX) hydrolyzed, reduced, and converted through the acid chloride to the cyclic ketone X; Clemmensen reduction of the ketone gives Diels' hydrocarbon. In the second synthesis,[18] β-(1-naphthyl)-ethylmagnesium bromide is reacted

[17] Bergmann and Hillemann, Ber., 66, 1302 (1933); Hillemann, Ber., 68, 102 (1935); 69, 2610 (1936); cf. Diels and Rickart, Ber., 68, 325 (1935).
[18] Harper, Kon, and Ruzicka, J. Chem. Soc., 124 (1934).

with 2,5-dimethylcyclopentanone to give an alcohol (XI) which after dehydration with phosphorus pentoxide is cyclized to yield the hydrocarbon XII. Selenium dehydrogenation of XII gives 3′-methyl-1,2-
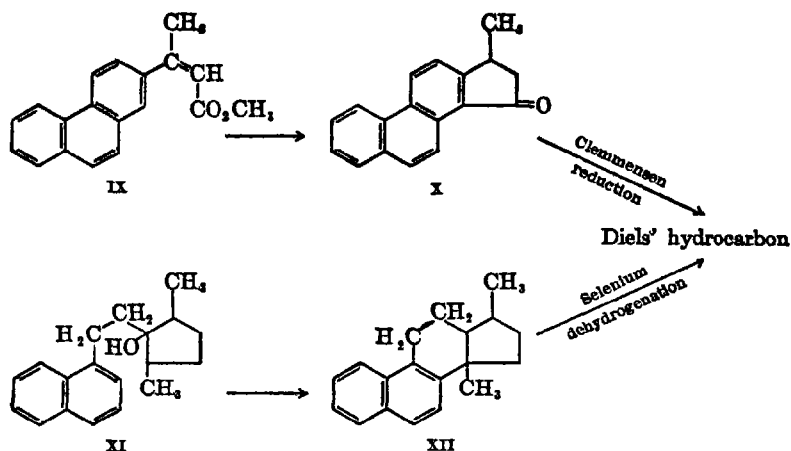
cyclopentenophenanthrene. Identification of the end product with the sterol hydrocarbon had to be made by an elaborate series of physical measurements, as well as by means of the picrate and other addition products, because the hydrocarbon does not give melting-point depression when mixed with structurally similar compounds. Diels' hydrocarbon as obtained from sterols by dehydrogenation has a magnificent blue fluorescence, which is absent in the synthetic product. Both preparations react with bromine to give a well-defined tribromide,[19] and with nitrous acid to form an isonitroso compound of uncertain structure. The characterization of this hydrocarbon is of great importance, for its formation by selenium dehydrogenation serves as one of the most convenient ways of discovering new members of the steroids.

The ring enlargement that takes place with selenium dehydrogenation at temperatures above 400° has attracted some interest. Model experiments on the α- and β-methyl- and ethyl-hydrindenes show that they undergo ring enlargement to produce naphthalene or methylnaphthalene at temperatures of 450°, but not at lower temperatures.[20] The absence of ring enlargement at lower temperatures has been confirmed by other model experiments on several related hydrindenes.[21] Diels'

[19] Diels and Rickert, *Ber.*, **68**, 267 (1935).

[20] Ruzicka and Peyer, *Helv. Chim. Acta*, **18**, 676 (1935).

[21] Clemo and Dickenson, *J. Chem. Soc.*, **735** (1935); Chuang, Ma, and Tien, *Ber.*, **68**, 1946 (1935).

hydrocarbon, however, does not give chrysene when treated with selenium or palladium at 450°.

Methylcholanthrene. The formation of Diels' hydrocarbon from the sterols and the bile acids suggests the nature of the nucleus, but the extremely poor yields obtained by dehydrogenation weaken the proof that the nucleus is cyclopentanoperhydrophenanthrene. Two of the bile acids, however, can be converted to methylcholanthrene in relatively good yield. Since the structure of methylcholanthrene can be established by degradation and by synthesis, this transformation materially strengthens the proof of the nature of the nucleus.

From cholic or desoxycholic acid, 12-ketocholanic acid (XIII) is obtained by methods which will be discussed later (p. 1363). Pyrolysis of the ketocholanic acid gives the hydrocarbon dehydronorcholene (XIV), and selenium dehydrogenation of the latter produces methylcholanthrene in a yield of 30 per cent.[22] On oxidative degradation methylcholanthrene is converted to 5,6-dimethyl-1,2-benzanthraquinone (XVI), which, in turn, is characterized by further oxidation to 1,2,5,6-anthraquinonetetracarboxylic acid.[23] In the synthesis[24] of methylcholanthrene a five-membered ring is formed on p-bromotoluene, giving a bromomethylhydrindene. The Grignard (p. 500) compound (XVIII) from this hydrindene is then reacted with α-naphthoyl chloride (XVII) to give a ketone (XIX) which on pyrolysis yields methylcholanthrene. By this transformation and synthesis, not only is the presence of a five-membered ring in appropriate sequence to three six-membered rings shown, but the attachment of the principal side chain of the bile acids at C_{17} is also established.

Relationship of the Hydroxyl Group and Double Bond in Cholesterol. The hydroxyl group and the double bond in cholesterol (II) are the chief points of attack in its degradation. By examination of the oxidation products these two functions have been found to be present in two different rings in an α,γ-system, the hydroxyl group being located at C_3 and the double bond at $C_5 : C_6$. The evidence follows:

1. Nitration of cholesteryl acetate yields a nitrocholesteryl acetate in which the nitro group is presumably attached at C_6.[25] Reduction of the nitro compound with zinc and acetic acid yields, with elimination of the nitro group, 3-hydroxy-6-ketocholestane.[26] Oxidation of the latter

[22] Cook and Haslewood, *J. Chem. Soc.*, 428 (1934).

[23] Wieland and Wiedersheim, *Z. physiol. Chem.*, 186, 229 (1930); Wieland and Dane, *ibid.*, 219, 240 (1933); Cook and Haslewood, *J. Soc. Chem. Ind.*, 52, 758 (1933); Cook, Hewett, and Haslewood, *ibid.*, 52, 949 (1933).

[24] Fieser and Seligman, *J. Am. Chem. Soc.*, 57, 228, 942 (1935).

[25] Mauthner and Suida, *Monatsh.*, 15, 85 (1894); 24, 648 (1903).

[26] Windaus, *Ber.*, 36, 3752 (1903).

gives rise through the stages of cholestanedione (XXIII) and a ketodi-carboxylic acid to the tetracarboxylic acid (XX).[27] In this degradation, ring A is opened first and then ring B. By a somewhat different pro-cedure—conversion of cholestanonol to chlorocholestanone—ring B may

XIII
12-Ketocholanic acid

XIV
Dehydronorcholene

XV
Methylcholanthrene

XVIII

+

XVII
α-Naphthoyl chloride

XIX

XVI
5,6-Dimethyl-
1,2- benzanthraquinone
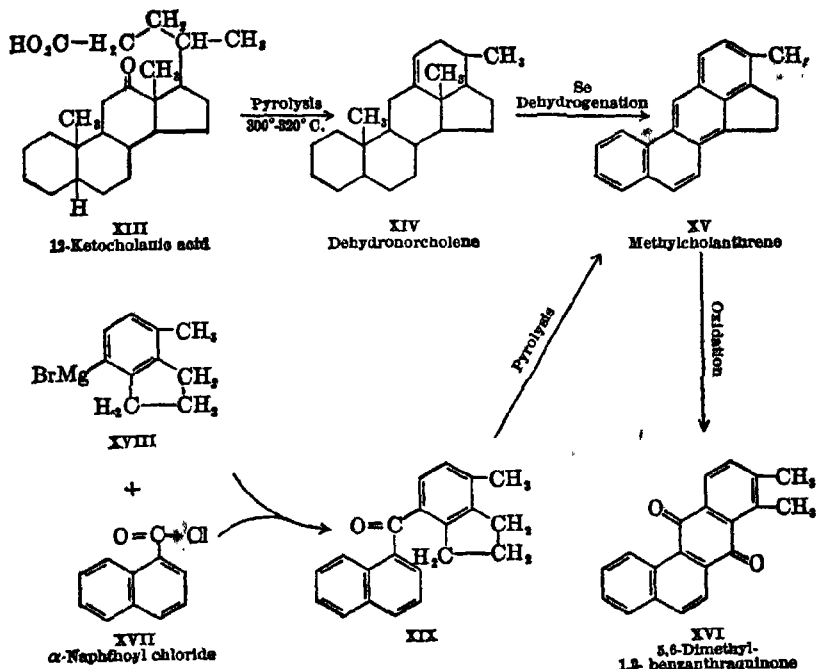
be opened first and then ring A.[28] With either procedure the same tetra-carboxylic acid (XX) results. Assuming that no rearrangements occur, the above transformations indicate that the hydroxyl group and the double bond are contained in separate rings.

2. Oxidation of cholesterol by potassium permanganate, hydrogen peroxide, or perbenzoic acid yields two isomeric cholestanetriols, $C_{27}H_{48}O_3$ (XXI),* which on further oxidation give two isomeric hydroxy-diketones, $C_{27}H_{44}O_3$ (XXII).[29] Dehydration and reduction convert both these ketones to the same cholestanedione (XXIII). The proper-ties of this dione are those of a γ-diketone. It reacts with hydrazine to

[27] Windaus and v. Staden, Ber., 54, 1059 (1921).

[28] Windaus and Stein, Ber., 37, 3699 (1904).

* Permanganate produces a cis, hydrogen peroxide a trans, configuration. Cf. Ellis and Petrow, J. Chem. Soc., 1078 (1939).

[29] Windaus, Ber., 40, 257 (1907); Pickard and Yates, J. Chem. Soc., 93, 1678 (1908); Westphalen, Ber., 48, 1064 (1915); Criegee, Ber., 65, 1770 (1932).

form a pyridazine;[30, *] the ketodicarboxylic acid formed from the diketone by oxidation is very stable and definitely not a β-keto acid, since the corresponding hydroxy acid obtained by reduction of the carbonyl

II. Cholesterol

XX

Oxidation and rearrangement

H₂O₂ or KMnO₄

XXI
Cholestanetriol

XXII
Cholestanedionol

Dehydration and reduction

XXIII
Cholestanedione

XXIV
Cholestenone

O₂ or KMnO₄

XXV

XXVI

Clemmensen reduction

Double Barbier-Wieland degradation

XXVII

XXVIII

[30] Windaus, Ber., **39**, 2249 (1906).

* According to the measurements of Noller, J. Am. Chem. Soc., **61**, 2975 (1939), this pyridazine has a molecular weight several times that of theory and may well be a linear polymer. Bursian, Ber., **73**, 922 (1940), has repeated Noller's work and finds a molecular weight of 1.25–1.5 times theory. These contradictory claims somewhat weaken the argument from pyridazine formation that cholestanedione is a γ-diketone.

group readily lactonizes.[31] These reactions show that the hydroxyl and double bond form an α,γ-system.

3. Cholestenone (XXIV) is formed from cholesterol by the action of copper oxide at 290°;[32] better, by cold, two-phase oxidation of cholesterol dibromide followed by debromination with zinc[33] or sodium iodide;[34] and best by treatment of cholesterol with aluminum tertiary alkoxides in the presence of a large excess of a ketone like acetone.[35] Cholestenone has an absorption spectrum (max. 240 mμ) which indicates that the carbonyl group and double bond form a conjugated system.[36] When cholestenone is oxidized with ozone or potassium permanganate, two products result: an acid of composition $C_{27}H_{44}O_4$ (XXV), and, as the principal product, a keto acid, $C_{26}H_{44}O_3$ (XXVI), formed with the loss of carbon dioxide.[37] The production of these two acids is satisfactorily explained only if a structure with the carbonyl and the double bond in the same ring is assigned to cholestenone.[38] It is evident, then, that cholestenone is formed from cholesterol by oxidation of the hydroxyl to a carbonyl group and a shift of the double bond from one ring to another. A rearrangement of the double bond attached to a carbon atom (C_5) common to both rings offers the simplest explanation of the transformation.

4. Reduction of the carbonyl group of the keto acid $C_{26}H_{44}O_3$ (XXVI) by the Clemmensen method gives the acid $C_{26}H_{46}O_2$ (XXVII). This acid may be degraded stepwise by a method (Barbier-Wieland degradation) that in effect counts the methylene groups following a carboxyl group.* The steps involved are:

$$R-CH_2-CO_2Et \xrightarrow{R'MgBr} R-CH_2-\underset{\underset{H}{\overset{\displaystyle |}{\text{OH}}}}{C}=(R')_2 \xrightarrow{CrO_3} \quad$$

$$\downarrow -H_2O$$

$$R-\underset{\underset{H}{|}}{C}=C=(R')_2 \xrightarrow{O_3 \text{ or } CrO_3} \quad \to R-CO_2H$$

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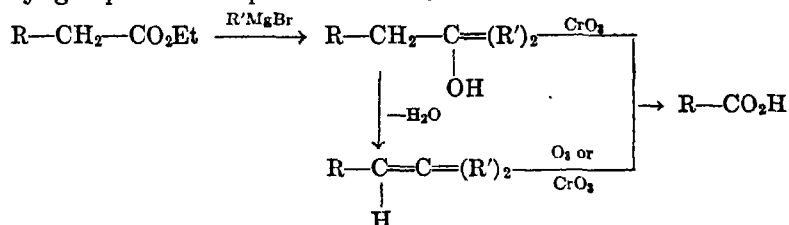
[31] Windaus and Hossfeld, Z. physiol. Chem., 145, 177 (1925).

[32] Diels and Abderhalden, Ber., 37, 3092 (1904); Windaus, Ber., 39, 518 (1906).

[33] Windaus, Ber., 39, 518 (1906); Ruzicka, Brüngger, Eichenberger, and Meyer, Helv. Chim. Acta, 17, 1407 (1934).

[34] Schoenheimer, J. Biol. Chem., 110, 461 (1935).

[35] Oppenauer, Rec. trav. chim., 56, 137 (1937).

[36] Menschick, Page, and Bossert, Ann., 495, 225 (1932); Mohler, Helv. Chim. Acta, 20, 289 (1937).

[37] Lettré, Z. physiol. Chem., 221, 73 (1933).

[38] Bonstedt, ibid., 214, 173 (1933); cf. reference 39.

* This method of degradation was originally developed by Barbier and Locquin, Compt. rend., 156, 1443 (1913), as a general process for degrading acids. It was later employed by Wieland, e.g., Wieland, Schlichting, and Jacobi, reference 46, but apparently without knowledge of Barbier's earlier work. Although the method is usually referred to as the Wieland degradation, it seems more appropriate to designate it as the Barbier-Wieland degradation.

By this means the acid XXVII after two degradations yields the acid $C_{24}H_{42}O_2$ (XXVIII), which cannot be degraded further;[39] thus, two methylene groups are diagnosed. The product (XXVIII) yields carbon monoxide easily when heated with concentrated sulfuric acid, and forms esters with difficulty; this indicates that the carboxyl group is attached to a quaternary carbon. Since the carboxyl group of the keto acid (XXVI) originated from the carbonyl of cholestenone, the position of the hydroxyl group of cholesterol is placed as being three carbons removed from this quaternary carbon, i.e., at C_3.

The Size of Rings A and B. The size of rings A and B has been determined by examination of the dicarboxylic acids produced by opening each ring separately. According to Blanc's rule,[40] when dicarboxylic acids are heated with acetic anhydride and distilled, or simply distilled (thermal decomposition), ketones result from 1,6- or 1,7-dicarboxylic acids while 1,4- and 1,5-diacids produce anhydrides.

Dihydrocholesterol (XXIX), the product of catalytic hydrogenation of cholesterol at room temperature, is readily oxidized to a dicarboxylic
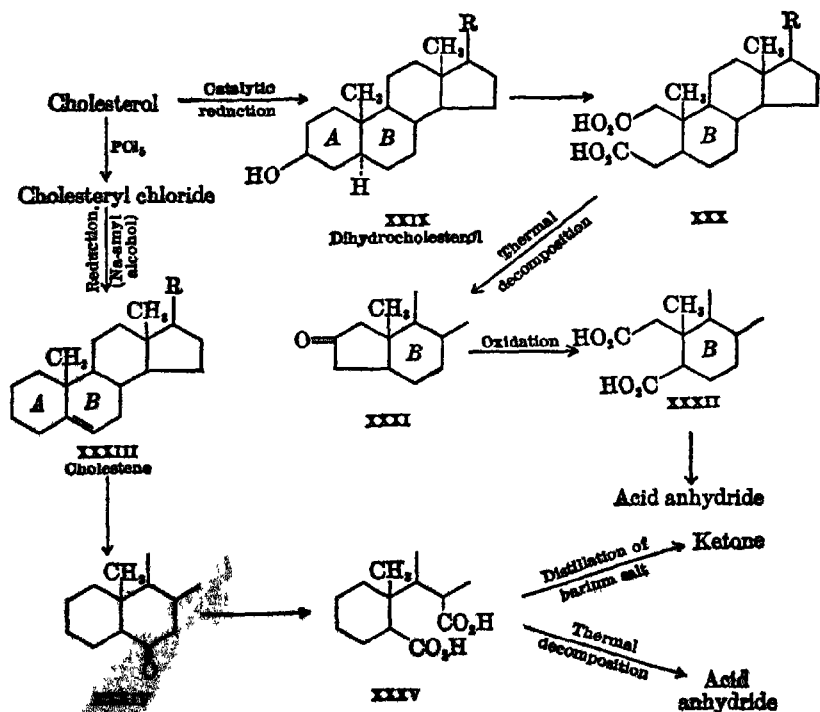
[39] Tschesche, *Ann.*, **498**, 185 (1932).
[40] Blanc, *Bull. soc. chim.*, [3] **33**, 893 (1905).

acid, $C_{27}H_{46}O_4$ (XXX). When subjected to thermal decomposition, the latter yields, with loss of water and carbon dioxide, a cyclic ketone, $C_{26}H_{44}O$ (XXXI). This ketone on oxidation gives rise to another dicarboxylic acid, $C_{26}H_{44}O_4$ (XXXII), but from this diacid the acetic anhydride treatment produces an acid anhydride and not a ketone.[41] Ring A, which is opened in the oxidation, is clearly six-membered.

When the ring containing the double bond is opened in a similar manner, somewhat different results are obtained.[42] Cholesterol is converted to cholestene (XXXIII) by reducing cholesteryl chloride with sodium and amyl alcohol. Nitration and reduction form a ketone, heterocholestanone (XXXIV), which, on oxidation, is converted into a dicarboxylic acid, $C_{27}H_{46}O_4$ (XXXV); but when this dicarboxylic acid is subjected to thermal decomposition, it forms an anhydride and not a ketone. The formation of the anhydride from the diacid was interpreted for many years as proof that ring B was a five-membered ring (structure IIa, ring II). Reëxamination of the situation has disclosed a number of loopholes. In the first place, it has been shown that substituted adipic acids do not always behave as might be expected;[43] and, secondly, Stange[44] has found that the barium salt of the diacid (XXXV) does form a ketone. The discrepancy between fact and theory in this case has led Wieland and Dane[10] to modify Blanc's rule (pp. 81, 1358) to apply only to those compounds in which the carboxyl groups are attached to the same ring; for example, the dicarboxylic acids formed by opening rings B and C (see structure I) would be expected to behave anomalously.*

From another series of reactions supplementary evidence as to the nature of ring B may be obtained.[45] Oxidation of cholesterol with hypobromite converts it to an unsaturated dicarboxylic acid, $C_{27}H_{44}O_4$ (XXXVI, Diels' acid), which may be progressively oxidized through a keto diacid (XXXVII), a diketo triacid (XXXVIII), to a tricarboxylic acid, $C_{25}H_{42}O_6$ (XXXIX). When subjected to thermal decomposition the tricarboxylic acid loses carbon dioxide and water and forms a keto-monocarboxylic acid, $C_{24}H_{40}O_3$ (XL). Opening of the newly formed

[41] Windaus and Dalmer, Ber., 52, 162 (1919); Windaus, Rosenbach, and Riemann, Z. physiol. Chem., 130, 113 (1923).

[42] Windaus and Dalmer, reference 41; Windaus, Ber., 53, 488 (1920).

[43] Farmer and Kracovski, J. Chem. Soc., 680 (1927). Cf. Hill, J. Am. Chem. Soc., 52, 4110 (1930).

[44] Stange, Z. physiol. Chem., 213, 74 (1933).

* Model studies on four of the six theoretically possible perhydrodiphenic acids by Vocke, Ann., 508, 1 (1934), cf. Hückel, ibid., 508, 10 (1934), and by Linstead and Walpole, J. Chem. Soc., 850 (1939), do not clarify the situation, since all the acids yield anhydrides, and three give cyclic ketones. The spatial configuration of the carboxyl groups is perhaps the deciding factor [Ruzicka, Furter, and Thomann, Helv. Chim. Acta, 16, 327 (1933)].

[45] Diels and Abderhalden, Ber., 36, 3179 (1903); Windaus, Ber., 41, 611, 2558 (1908); 42, 3770 (1909); 45, 1316, 2421 (1912).

ring by oxidation gives a tricarboxylic acid, $C_{24}H_{40}O_6$ (XLI). This acid likewise forms a keto acid (XLII) when treated with acetic anhydride, thus demonstrating a 1,6-dicarboxylic acid. Since in the transformation to the tricarboxylic acid (XLI) three carbon atoms are lost as carbon dioxide, and the end product can be converted to a keto acid, both rings A and B must have been six-membered.

The Size of Ring D. Dehydration of any of the bile acids is readily effected by distillation in high vacuum. The resulting unsaturated acids may be catalytically hydrogenated to the parent cholanic acid
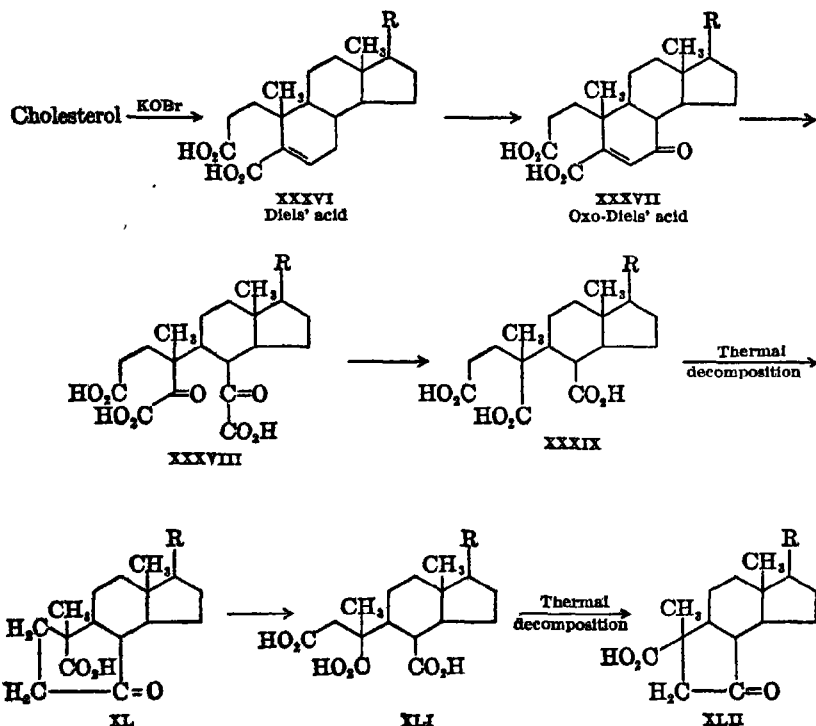
(XLIII). By means of the Barbier-Wieland degradation and oxidation, cholanic acid can be degraded stepwise [46] through the following stages: cholanic acid (C_{24}) → norcholanic acid (C_{23}) → bisnorcholanic acid (C_{22}, XLIV) → etiocholyl methyl ketone* (C_{21}, XLV) → etiocholanic

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[46] Wieland, Schlichting, and Jacobi, Z. physiol. Chem., **161**, 80 (1926).

* Etiocholyl methyl ketone was not obtained by Wieland by degradation but as a by-product from the oxidation mixtures of the previous steps. Bisnorcholanic acids have been degraded to etiocholyl methyl ketones by a number of other workers, however. See Shimizu and Kazuno, Z. physiol. Chem., **244**, 167 (1936), and Morsman, Steiger, and Reichstein, Helv. Chim. Acta, **20**, 1 (1937). The data of Reichstein show that 100 g. of cholic acid yields about 40 g. of norcholic acid and, by succeeding degradation, 10 g. of bisnorcholic acid and 1.5 g. of trihydroxyetiocholyl methyl ketone.

acid (C_{20}, XLVI) → etiocholanone (C_{19}, XLVII) → etiobilianic acid (C_{19}, XLVIII, a dicarboxylic acid). These reactions demonstrate the presence of a side chain, $-\overset{\overset{\textstyle CH_3}{|}}{C}H-CH_2-CH_2-CO_2H$, attached to a ring.

XLIII
Cholanic acid

XLIV
Bisnorcholanic acid

XLV
Etiocholyl methyl ketone

XLVI
Etiocholanic acid

XLVII
Etiocholanone

XLVIII
Etiobilianic acid
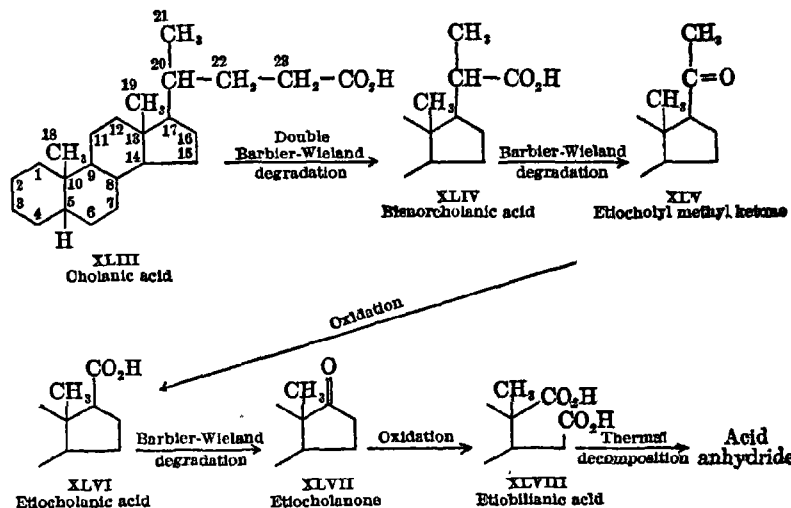
The transformation of etiocholanone to etiobilianic acid without loss of carbon shows that this attachment is through a tertiary carbon, and that adjacent to the tertiary carbon there is a methylene group. Thermal decomposition of the end product of oxidation, etiobilianic acid, gives an acid anhydride and not a ketone. Since the ring is opened without loss of carbon, and with the production of an acid that behaves like glutaric acid, a five-membered ring is indicated. Because of the failure of Blanc's rule, the formation of an anhydride is not adequate proof that ring D is five-membered. When the evidence from the dehydrogenation experiments, particularly the formation of methylcholanthrene, is added to this degradation, the proof is convincing.

The Degradation of Lithocholic Acid. When lithocholic acid (XLIX), 3(α)-hydroxycholanic acid, is oxidized with nitric acid, the ring bearing the hydroxyl group is opened to give two isomeric tricarboxylic acids, lithobilianic (L) and isolithobilianic (LI) acids, formed by the rupture of bonds on different sides of the hydroxyl group.[47] These two acids are identical with a pair formed by stepwise oxidation of coprosterol. In the first step, ring A is opened with the formation of two dicarboxylic acids, one of which is less soluble than the other. Further oxidation removes the isopropyl group of the isoöctyl side chain,

[47] Wieland and Weyland, Z. physiol. Chem., **110**, 123 (1920).

with the production of lithobilianic acid from the less soluble dicarboxylic acid and isolithobilianic acid from the other acid.[45] As would be expected, thermal and oxidative degradation proceed through stages analogous to those described for cholesterol (XXXVI–XLII). The transformation is summarized in structures LII–LV. The end product, the

CH$_3$
HO$_2$C
HO$_2$C *A* *B*
H

LI. Isolithobilianic acid

CH$_3$
CH–CH$_2$–CH$_2$–CO$_2$H
CH$_3$

CH$_3$
CH$_3$ 9 8
2 10 B
A 5 7
12 4 6
HO
H

XLIX. Lithocholic acid

CH$_3$
A *B*
HO$_2$C
HO$_2$C H

L. Lithobilianic acid

CH$_3$
B
O=C

LII

CH$_3$
HO$_2$C O

LIII

CH$_3$
3 5 7
HO$_2$C CO$_2$H CH$_2$–CO$_2$H

LIV

C$_4$H$_8$CO$_2$H
CH$_3$
2 5 7
HO$_2$C
HO$_2$C CO$_2$H

LV

C$_4$H$_8$CO$_2$H
CH$_3$
5 CH$_3$ 9 8
7
HO$_2$C 1
HO$_2$C—C=O

LVI

C$_4$H$_8$CO$_2$H
CH$_2$
CH$_3$
HO$_2$C— H CO$_2$H

LVII

CH$_3$
CH(CH$_3$)$_2$CH CH$_3$ CH$_3$
CH$_3$
CH$_3$
2 1
HO$_2$C
HO$_2$C CO$_2$H

XLI
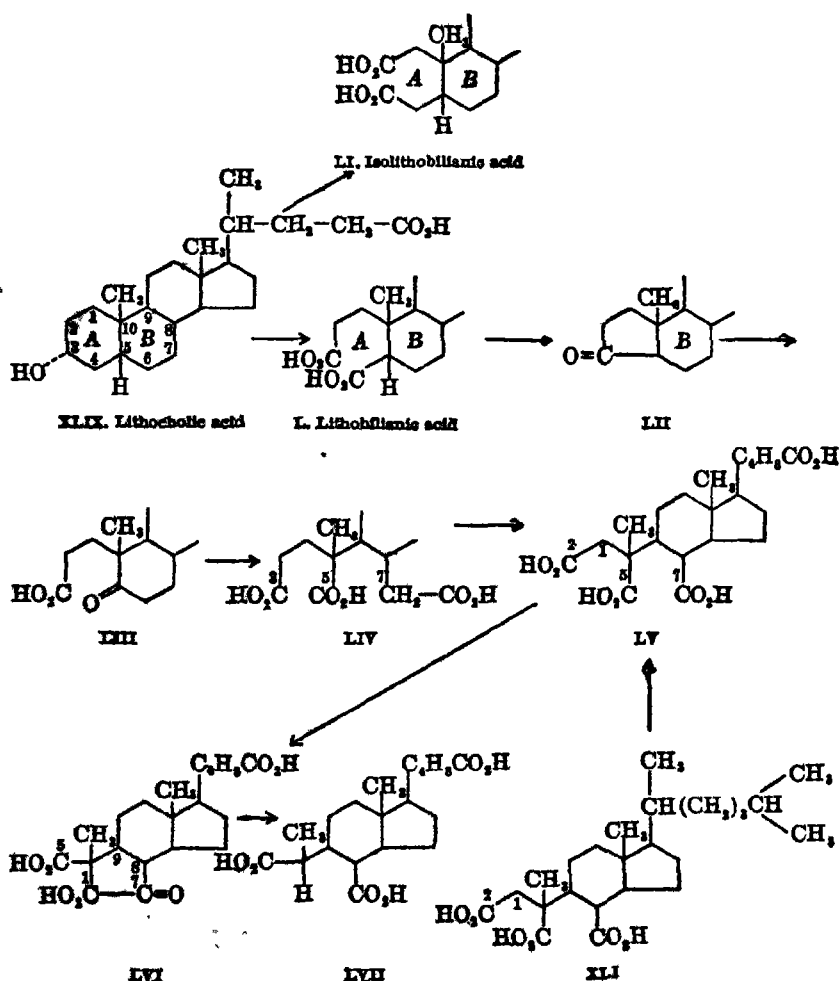
tetracarboxylic acid, C$_{21}$H$_{32}$O$_8$ (LV), is identical with an acid obtained by Windaus[46] from the oxidation of the tricarboxylic acid (XLI) formed in the degradation of cholesterol. Thermal decomposition of LV

[45] Langer, ibid., 216, 139 (1933). Cf. Windaus and Riemann, ibid., 126, 277 (1923).

[46] Cf. Wieland, Dane, and Scholz, ibid., 211, 261 (1932).

produces a pyroketodicarboxylic acid (LVI), thus showing the presence of an adipic acid system. This keto acid, when oxidized, passes through the stage of a malonic acid to a tricarboxylic acid (LVII) from which only an anhydride can be formed. The end product must contain a glutaric acid system, while the malonic acid from which it was formed demonstrates a branching of the chain. On inspection it is apparent that the branching takes place at the quaternary carbon atom diagnosed in the degradation of cholestenone. Production of the ketodicarboxylic acid (LVI) constitutes further proof that ring B is six-membered, for otherwise anhydride formation would occur.

The Degradation of Desoxycholic Acid. Chromic acid oxidation in the cold of desoxycholic acid (LVIII) converts it to the corresponding diketo acid, dehydrodesoxycholic acid (LIX); further oxidation by means of nitric acid opens up one ring to form desoxybilianic acid, a ketotricarboxylic acid (LX), which by Wolff-Kishner reduction* is converted to lithobilianic acid (L).[50] One of the hydroxyl groups of desoxycholic acid must, therefore, be attached at C_3 as in lithocholic acid and cholesterol. If dehydrodesoxycholic acid (LIX) is treated with zinc and alcoholic hydrochloric acid, the 3-carbonyl group is reduced to methylene, with the formation of 12-ketocholanic acid (XIII), which is resistant to nitric acid oxidation. By bromination and subsequent hydrolysis, an hydroxyl group may be introduced on a carbon adjacent to the carbonyl. The resulting hydroxyketone is readily oxidized with loss of carbon to a ketodicarboxylic acid (LXIII). Repetition of this process gives rise to a ketotricarboxylic acid (LXVI) and finally to the acid $C_{13}H_{20}O_6$ (LXVIII).[50] The other fragment (LXVII) has not been isolated. The structure of the important triacid, $C_{13}H_{20}O_6$ (LXVIII), follows from another oxidative procedure.

The Structure of Acid $C_{13}H_{20}O_6$. When desoxycholic acid is acted upon by a mixture of concentrated nitric and sulfuric acids (mixed acids) in the cold, it passes through the stage of a diketodicarboxylic acid (LXIX) to give a tetrabasic acid, $C_{16}H_{24}O_8$ (LXXI), and 1,3,3-butanetricarboxylic acid (LXX) as a by-product. Thermal decomposition of the tetrabasic acid (LXXI) gives in low yield a pyroketodicarboxylic acid (LXXII) which on oxidation is converted through a malonic acid (LXXIII) to the acid $C_{13}H_{20}O_6$.[51] Clemmensen reduction (p. 644) of the pyroketone (LXXII) transforms it into a dicarboxylic acid. The diester of this dicarboxylic acid reacts with phenylmag-

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* Wolff-Kishner reduction: The reduction of a carbonyl group to methylene by heating the hydrazone or semicarbazone with sodium ethylate in ethyl alcohol at ca. 180° (p. 644).

[50] Wieland and Kuhlenkampff, Z. physiol. Chem., **108**, 295 (1920).

[51] Wieland and Schlichting, ibid., **134**, 276 (1924); Wieland and Vocke, ibid., **177**, 68 (1929).

nesium bromide in such a way that but one ester group is converted to a carbinol. Barbier-Wieland degradation of the carbinol

LVIII. Desoxycholic acid   LIX. Dehydrodesoxycholic acid   LX. Desoxybilianic acid

Wolff-Kishner reduction

Lithobilianic acid
(L)

XIII. 12-Ketocholanic acid   LXI   LXII

LXIII   LXIV   LXV

LXVI   LXVII   LXVIII
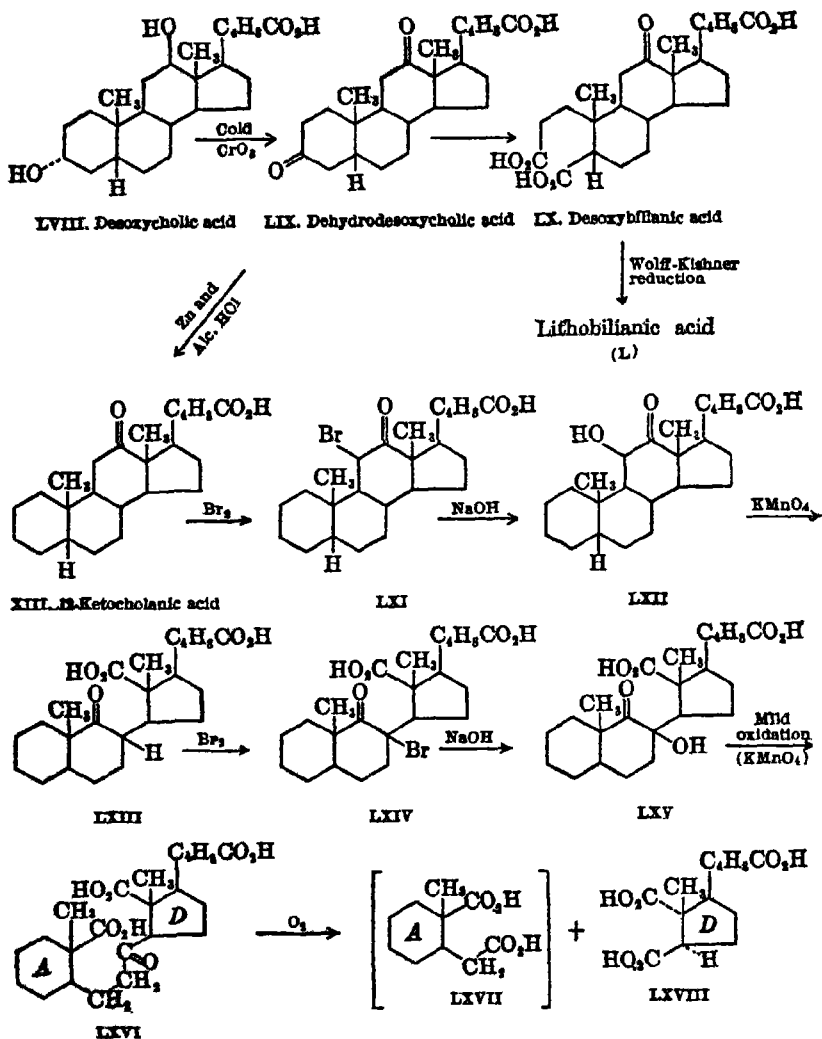
shows that the reactive carboethoxy group is present in the side chain

$$CH_3$$
$$|$$
—CH—CH$_2$—CH$_2$—CO$_2$H, found in desoxycholic acid.[4] This side chain must also be present in the acid C$_{13}$H$_{20}$O$_6$. Since the side chain is known to be attached to a five-membered ring, and three carboxyl groups may be detected by titration, only the fragment —CH$_3$ remains

to be placed. That this fragment is a methyl group attached at C_{13} follows from an ingenious argument of Wieland and Dane.[12]

The acid $C_{13}H_{20}O_6$ (LXVIII) readily forms an anhydride; saponification of the anhydride does not produce the original acid, but an isomer which has a lower melting point and greater solubility. Evidently a rearrangement from a *trans* to a *cis* form occurs, and if LXVIII has a *trans* structure, then the acid $C_{16}H_{24}O_8$ (LXXI) must have a *trans* structure. The low yield of pyroketone (LXXII) is thus accounted for, and an interesting question of isomerism is raised, for the ketone is evidently a decalin-like compound containing two cyclopentane rings in the *trans* position. Such a system has not been investigated, but according to Hückel[53] the *cis* form should be strain-free, while the *trans* modification should exhibit a moderate degree of strain (p. 114). The investigations of Windaus[54] have shown that when a five-membered ring

Desoxycholic
acid
LVIII
$\xrightarrow[\text{HNO}_3 + \text{H}_2\text{SO}_4]{\text{Cold}}$

LXIX
(*R = C₄H₈CO₂H)

LXX. 1,3,3-Butane-tricarboxylic acid

LXXI. Tetracarboxylic acid, $C_{15}H_{24}O_8$

LXXII. Pyroketo-dicarboxylic acid

LXXIII. Malonic acid

LXVIII Tricarboxylic acid, $C_{13}H_{20}O_6$
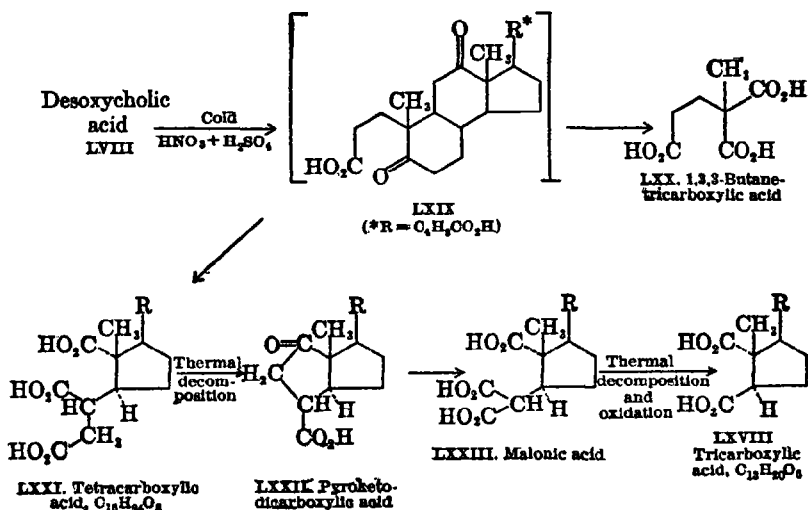
is formed on a cyclohexane ring by thermal decomposition of dicarboxylic acids a *cis* form results, and in no case where attachment is through a secondary ring carbon is the *trans* form produced. In the formation of the pyroketone, however, the *trans* configuration persists unchanged, rearrangement being prevented through the influence of some other group. A methyl group attached at C_{13} would exert such an influence, while if it were attached at C_{14} it would not prevent rearrangement.

[52] Wieland and Dane, *ibid.*, **216**, 91 (1933).

[53] Hückel, "Theoretische Grundlagen der organischen Chemie," Akademische Verlagsgesellschaft, Leipzig (1934), 2nd ed., Vol. I, p. 63.

[54] Windaus, Hückel, and Reverey, *Ber.*, **56**, 91 (1923); Windaus, *Ann.*, **447**, 233 (1926).

The production (LVIII–LXVIII) of the acid $C_{13}H_{20}O_6$ constitutes a proof that the second hydroxyl of desoxycholic acid is attached at C_{12}. Since the demonstrable five-membered ring comes through the oxidation unscathed, the second hydroxyl could not have been attached to it. Examination of the other dihydroxycholanic acids excludes the possibility of attachment to ring B. In ring C only two positions, C_{11} and C_{12}, can be considered, and only the latter is compatible with the behavior on bromination. For example, if XIII were 11-ketocholanic acid, the result of the first bromination (LXI–LXII) would be a tricarboxylic acid.

The other method (LXIX–LXXIII) of producing the acid $C_{13}H_{20}O_6$, though not affording as good direct evidence, serves as supplementary proof. If LXIX were an 11-keto compound, a $-CH_2-CO_2H$ group would be formed on C_{13} as one of several products of the oxidation. Actually the oxidation proceeds in relatively good yield to form acid $C_{16}H_{24}O_8$, and the behavior of this acid is such that other structures cannot be considered; the formation of the malonic acid LXXIII, for example, confirms the branching of the chain at C_8.

The Side Chains. The point of attachment, C_{17}, of the principal side chain to the nucleus was suggested from x-ray and surface-film measurements (pp. 1348, 1762),[8] and confirmed by the formation of methylcholanthrene from 12-ketocholanic acid.[22] The evidence on this point has been discussed and may be regarded as satisfactory.

After providing for the carbon and hydrogen requirements of the nucleus and the side chain of the sterols and bile acids, there remain two carbons and six hydrogens to be attached. These have been placed as methyl groups at C_{10} and C_{13}. The evidence indicating attachment of one of these at C_{13} is given above. For some time certain of the English school of investigators favored attachment at C_{14} rather than at C_{13}, since such a structure fitted in with a postulated biological formation of cholesterol from isoprene units (p. 72).[55] The argument collapsed, however, when applied to ergosterol and to stigmasterol, which are alkylated at C_{24}, and the position at C_{14} is no longer seriously considered.

Proof that the second methyl group is attached at C_{10} is much more direct. The by-product of the nitric acid oxidation of desoxycholic acid, the tricarboxylic acid $C_7H_{10}O_6$ (LXX), loses carbon dioxide when heated and forms α-methylglutaric acid. With this as a clue, synthesis has established the structure of LXX as 1,3,3-butanetricarboxylic acid.[4] The formation of this acid shows the presence of a quaternary carbon atom bearing a methyl group. Since C_{10} is known to be quaternary from the degradation of cholestenone, the proof of the attachment of a

[55] Symposium on Sterol Structure, *J. Soc. Chem. Ind.*, **52**, 10 (1933).

methyl group at this point seems to be conclusive, for the tricarboxylic acid must result from rings A and B in the oxidation of desoxycholic acid.

Stereochemistry *

In cholestane (LXXIV), the parent hydrocarbon of cholesterol and dihydrocholesterol, there are centers of asymmetry at C_5, C_8, C_9, C_{10}, C_{13}, C_{14}, C_{17}, and C_{20}. The number of possible stereoisomers is accordingly 2^8, or 256. With an hydroxyl group at C_3 the possible isomers are increased to 512. Coprostane (LXXV) is one of the stereoisomers of cholestane, differing only in the spatial arrangement of the C_5—H. Nearly all the members of the cyclopentanoperhydrophenanthrene

LXXIV. Cholestane
(trans, trans, trans)

LXXIVa

LXXV. Coprostane
(cis, trans, trans)

LXXVa
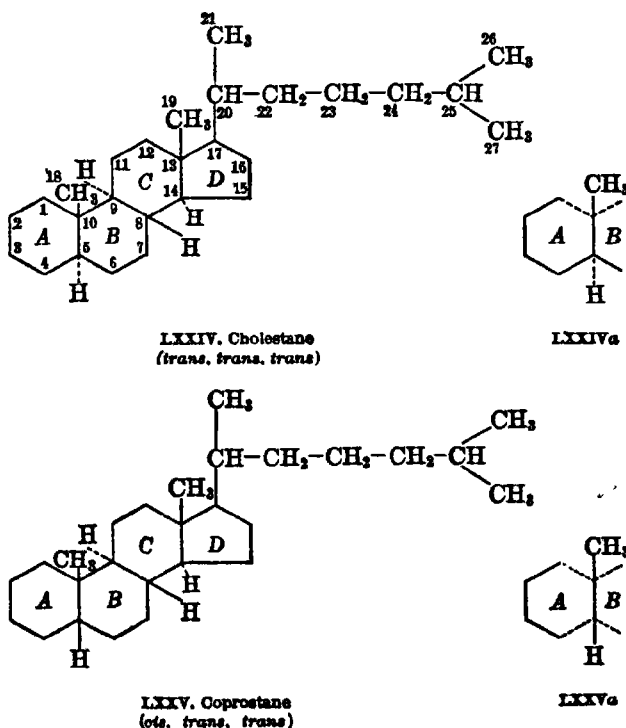
group are derivatives of these two hydrocarbons, and it would be valuable to have precise structures for both. Unfortunately, it is possible to portray only the probable structures and to indicate the inter-relationship.

* The several aspects of stereochemistry are considered in Chapter 4, p. 332.

The space models for the cholestane type (Fig. 1) and the coprostane type (Fig. 2) are suggested by similar representations by Ruzicka.[56] The models are built up from the assumption that the C_{10}—CH_3 (R) group in both structures projects out from the plane of the paper. The size of the balls does not show the probable atomic size, nor are the interatomic distances correctly represented; besides these defects, the position in space of the angular substituents and the relationship of one portion of the molecule to another may be entirely different from those shown. Nevertheless, the models are helpful in understanding the adjustments that probably occur in the change from one type to the other. Assuming that the C_{10}—CH_3 comes forward, then in the cholestane type the C_5—H goes into the plane of the paper, and in the
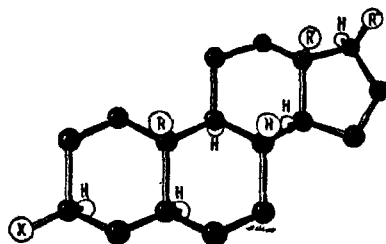
FIG. 1.—Cholestane type
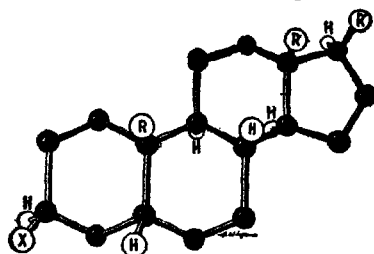
FIG. 2.—Coprostane type

The illustrations are reproductions of space models of the two types. The position in space of the atoms is shown by the size and the shading of the balls that are used to represent the various atoms or groups. The atoms nearest the eye are shown by the largest balls, and, where these represent carbon atoms, by full intensity of black. The atoms in planes below this are represented by smaller balls and by decreased intensity of black. The circles marked "X" represent any group attached at C_3.

coprostane type it comes out from the plane of the paper. The spatial adjustments in the configuration of rings A and B are such that in cholestane these two rings are "chair types"; in coprostane, "saddle

[56] Ruzicka, Furter, and Thomann, *Helv. Chim. Acta*, 16, 331 (1933).

types" (p. 114). From the models it can be seen that a substituent at C_3 does not occupy exactly the same relative position either to the C_5—H or to the C_{10}—CH_3 in both types. Aside from these differences the two molecules appear to be the same. The evidence presented below does not disclose any discrepancies in these representations, but the methods used to obtain it are not completely satisfactory.

For representations on a plane surface, dotted and solid lines are employed to indicate the spatial configuration. As has been mentioned previously, the dotted lines indicate bonds going into the plane of the paper; the solid lines, bonds lying in or coming out of the plane of the paper. The use of such lines (as is shown in structures LXXIV*a* and LXXV*a*) for an entire structure is cumbersome, and in practice only selected portions of the molecule are so represented. The method has the disadvantage that a solid line is the normal way of representing a linkage, and where the spatial configuration is unknown the implied structure may be erroneous.

Spatial Isomerism of the Nuclear Rings. The experimental evidence in support of the structures of cholestane and coprostane has been obtained largely from the chemical behavior of degradation products of the bile acids and from physical measurements on the hydrocarbons themselves.

Rings A/B. Windaus[57] has studied the behavior of the four lithobilianic acids when subjected to thermal decomposition. Lithobilianic acid (L) and *allo*lithobilianic acid (LXXVI) give the same pyro acid (LII); isolithobilianic acid (LI) and *allo*isolithobilianic acid (LXXVII) give two different pyro acids, LXXVIII and LXXIX. Clemmensen reduction of pyrolithobilianic acid and of pyroisolithobilianic acid gives the same desoxo compound (LXXX).* Lithobilianic acid and isolithobilianic acid must have the C_5—H and the C_{10}—CH_3 in the same relationship, since both are produced from lithocholic acid and both can be transformed to the same end product. *Allo*lithobilianic acid and *allo*isolithobilianic acid give different pyro acids and desoxopyro acids. Obviously, *allo*lithobilianic acid must have undergone a rearrangement in the thermal treatment, and the same arguments apply here as were used previously in the case of the acid $C_{13}H_{20}O_5$ (LXVIII) (p. 1363), that a rearrangement from a *trans* to a *cis* structure occurs. Since the lithobilianic acids may be regarded as degradation products of coprostane in which ring A has been opened, and the *allo*lithobilianic acids

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[57] Windaus, *Ann.*, **447**, 240 (1926), and reference 54.

*Allolithobilianic acid and *allo*isolithobilianic acid may be prepared from hyodesoxycholic (3,6-dihydroxycholanic) acid or from cholesterol. The transformation is discussed later (p. 1420).

as degradation products of cholestane, it follows that rings A/B have a *cis* relationship in coprostane and a *trans* relationship in cholestane. Similar results have been obtained by Lettré[47] with the corresponding dicarboxylic acids from dihydrocholesterol and coprosterol. Not only do these transformations establish the relationship of rings A/B, but also they show that aside from these rings the spatial configuration of the two types is the same.

L. Lithobilianic acid m.p. 279°   LXXVI. *Allo*litho-bilianic acid m.p. 212°   LI. Isolithobilianic acid m.p. 261°   LXXVII. *Allo*iso-lithobilianic acid m.p. 239°-240°

LII. Pyrolithobilianic acid   LXXX.   LXXVIII. Pyroiso-lithobilianic acid   LXXIX. Pyro*allo*iso-lithobilianic acid
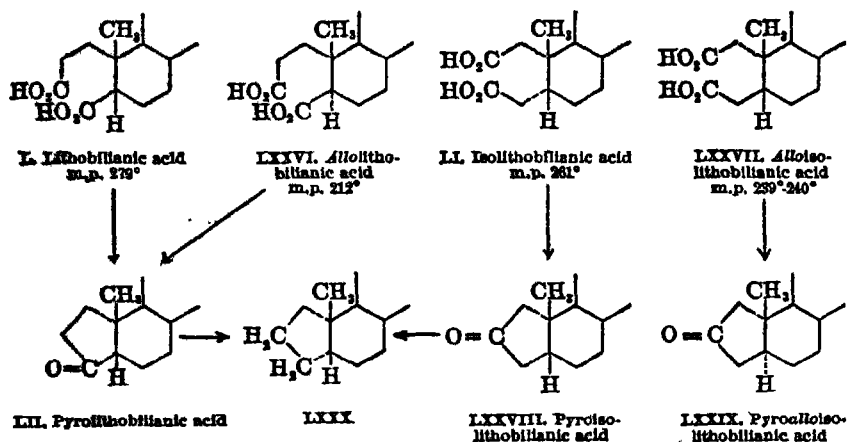
Ruzicka[58] has examined the physical constants of cholestane (LXXIV) and coprostane (LXXV), and compared them with the known examples of the *cis* and *trans* decalins. Cholestane was found to have a lower density, higher molecular refraction, and higher melting point than coprostane. Reasoning from the decalins (p. 484), the relationship of rings A/B is *trans* in cholestane and *cis* in coprostane.

In addition to the differences in the physical properties in the two structures, there are chemical dissimilarities. When the 3-keto compounds of the cholestane type are oxidized to acids, ring opening takes place principally at C_2—C_3, while with the bile acids which are of the coprostane type opening of ring A occurs chiefly at C_3—C_4.[59] The dicarboxylic acids formed by opening the ring between C_2 and C_3 are more soluble than those resulting from the cleavage of the C_3—C_4 bond. On treating the 3-keto compounds with bromine, substitution takes place predominantly at C_4 with the bile acids (coprostane type), and at C_2 with the cholestane structure.[60] These reactions suggest that enolization is primarily from C_4 to C_3 in one case, and C_2 to C_3 in the other.

[58] Ruzicka, Furter, and Thomann, *Helv. Chim. Acta*, 16, 327 (1933).
[59] Wieland, Dane, and Martius, *Z. physiol. Chem.*, 215, 15 (1933).
[60] Summary: Butenandt, Schramm, Wolff, and Kudszus, *Ber.*, 69, 2779 (1936). *Cf.* Ruzicka, Bosshard, Fischer, and Wirz, *Helv. Chim. Acta*, 19, 1147 (1936).

Marker,[61] however, has pointed out that these generalizations do not apply to 3-ketocoprostane itself, and argues that the substituent at C_{17} influences the course of oxidation and of substitution reactions on ring A.*

Rings B/C. The x-ray and surface-film measurements of Bernal[8] show that the molecules of the sterols must be flat, as in paraffin hydrocarbons with methyl side chains. Models of the sterol molecules in which rings B/C are *trans* are flat, in agreement with the physical measurements, while a *cis* structure at this point gives a bowed-in or condensed model. Another positive argument for such a configuration comes from the work of Wieland.[62] After 7,12-diketocholanic acid is heated for ten hours with dilute alkali, it can be recovered unchanged. Since the neighboring center of asymmetry to C_{12} carries a methyl group, rearrangement at this point is impossible, and the absence of rearrangement must mean that the C_8—H is in the stable *trans* configuration with respect to the C_9—H. This is in accord with Hückel's[68] experience that only *cis*-decalones rearrange into *trans* when treated with alkali. For convenience rather than with reason, the C_9—H is usually represented as being *trans* to the C_{10}—CH_3, and the practice is frequently bulwarked by the argument of steric hindrance.

Rings C/D. The behavior of the acid $C_{13}H_{20}O_6$ (LXVIII) on thermal decomposition furnishes the evidence for a *trans* relationship of the C_{13}—CH_3 and the C_{14}—H.† The structural representation of this

LXVIII. *Trans* form
of acid, $C_{13}H_{20}O_6$
m.p. 187° $[\alpha]_D$-27.8

LXXXI. Anhydride

LXXXII. *Cis* form
of acid, $C_{13}H_{20}O_6$
m.p. 136° $[\alpha]_D$ 36.4
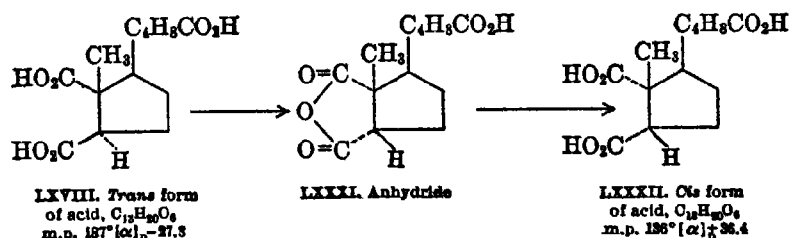
transformation, which was not given previously, is cited here. In the production of the anhydride LXXXI, a rearrangement occurs, and on saponification the lower-melting *cis* form, LXXXII, of the acid results.

[61] Marker *et al., J. Am. Chem. Soc.*, **61**, 3517 (1939).

* The interpretation of the experimental evidence by Marker has been questioned by Spring, *Ann. Repts. Chem. Soc. (London)*, **37**, footnote p. 358 (1940).

[62] Wieland and Wiedersheim, *Z. physiol. Chem.*, **186**, 232 (1930).

[63] Hückel, *Ann.*, **441**, 1 (1925). *Cf.* Windaus, Hückel, and Reverey, *Ber.*, **56**, 91 (1923); Linstead and Meade, *J. Chem. Soc.*, **935** (1934); Cook and Linstead, *ibid.*, **946** (1934); Barrett and Linstead, *ibid.*, **436** (1935).

† For a further argument indicating a *trans* configuration of rings C/D, see footnote on p. 1408.

The Side Chain at C_{17}. The ready formation of dehydronorcholene (XIV) from 12-ketocholanic acid (XIII) has been used by Wieland as an argument that the side chain at C_{17} occupies a *trans* position with respect to the C_{13}—CH_3. Consideration of space models hardly confirms this contention, and Ruzicka [64] has suggested that a *cis* configuration is a better representation. In Figs. 1 and 2, this side chain is shown in the *cis* configuration, but without convincing evidence the issue must be left open.

Spatial Isomerism of the Hydroxyl Groups. In this discussion the spatial configurations of the hydroxyl groups are described by the prefixes α and β following a convention introduced by Fieser.[65] A β-configuration is taken as that of the C_3—OH of cholesterol, dihydrocholesterol, etc., and an α-configuration as that of the same group in the bile acids. Hydroxyl groups located at other positions on the ring, and on the side chains, are similarly described by these prefixes. Provisionally a ring hydroxyl group with β-configuration may be regarded as having a *cis* relationship to the nearest angular methyl group, and in accord with this is represented by a solid line. Conversely, an α-configuration may be regarded as *trans* to the reference point and is represented by a broken line. The advantage of this terminology is apparent since it is not too definite and readily permits adjustment should later work show that the spatial relationships are other than those now implied. This terminology may be supplemented by another practice. The first member of the epimeric pair to be isolated or synthesized is regarded as having a normal (*n*-) structure, while the second, or unusual form, is described as an *epi* modification.[66] Thus, dihydrocholesterol may be described as 3(β)-hydroxycholestane, and its epimer as *epi*dihydrocholesterol, or 3(α)-hydroxycholestane.

Ruzicka [67] and co-workers use a somewhat different convention. According to their practice, the spatial configuration of an hydroxyl group is described as *c* (*cis*) or *t* (*trans*) to the nearest center of asymmetry. An hydroxyl group at C_3 is referred to the C_5—H, one at C_{17} to the C_{13}—CH_3, etc. This usage is somewhat unsatisfactory since in many of the steroids there is an ethenoid linkage at C_5, and it is necessary to refer a C_3—OH to the C_5—H of the corresponding saturated compound. By this practice the hydroxyl group in both cholesterol and dihydrocholesterol is described by Ruzicka as 3(*t*)-hydroxy-, although in choles-

[64] Ruzicka, Goldberg, and Wirz, *Helv. Chim. Acta*, **18**, 61 (1935).

[65] Fieser, "The Chemistry of Natural Products Related to Phenanthrene," Reinhold Publishing Corp., New York, (1937), 2nd ed., p. 399.

[66] *Cf.* Butenandt and Müller, *Ber.*, **71**, 191 (1938).

[67] Ruzicka *et al.*, *Helv. Chim. Acta*, **17**, 1395, 1407 (1934); **18**, 61 (1935); **19**, 99, 842 (1936); **20**, 1557 (1937); **21**, 498 (1938).

terol the reference hydrogen at C_5 is absent. Miescher and Fischer [68] have suggested that this difficulty may be surmounted by using the C_9—H as a reference point.

The C_3—OH. When a definite reference point is stated, certain general relationships should be inherent in the assigned structures. Unfortunately, the conclusions that are reached from most of the reactions of the C_3—OH are somewhat at variance with those arrived at in another way. This is brought out by considering the isomeric cholestanols and coprosterols.

Catalytic hydrogenation of cholesterol or cholestanone [69] (LXXXIII) in neutral solvents gives dihydrocholesterol [70] (cholestanol), while hydrogenation of cholestanone in acid media (acetic acid and hydrobromic acid, or butyl ether and hydrobromic acid) forms *epi*dihydrocholesterol (LXXXIV).[71] Applying the rule of v. Auwers-Skita [72] that neutral media favor the formation of *trans* modifications and that acid media lead to *cis* structures, dihydrocholesterol is 3(*trans*)-hydroxy, and *epi*dihydrocholesterol is 3(*cis*)-hydroxy with respect to the C_5—H. On catalytic hydrogenation of cholestenone (XXIV) in neutral media, coprostanone (LXXXV) is formed,[73] apparently in violation of v. Auwers-Skita's rule. Continued hydrogenation produces coprosterol (LXXXVI) in acid media and *epi*coprosterol (LXXXVII) in neutral media.[73] From this mode of formation the relationship of the C_3—OH to the C_5—H appears to be *cis* in coprosterol and *trans* in *epi*coprosterol. The conclusion seems warranted that dihydrocholesterol and *epi*coprosterol are transoid (lower energy content) while coprosterol and *epi*dihydrocholesterol are cisoid (higher energy content).*

In agreement with this conclusion, when the four sterols are epimerized by heating in alcoholic solution at 180° with sodium ethoxide, or by refluxing with aluminum isopropoxide in xylene, the compounds which seem to have transoid nature predominate over the cisoid in a ratio

[68] Miescher and Fischer, *J. Soc. Chem. Ind.*, **58**, 113 (1938).

[69] Bruce, *Org. Syntheses*, **17**, 43 (1937), John Wiley & Sons, New York.

[70] Bruce and Ralls, *Org. Syntheses*, **17**, 45 (1937), John Wiley & Sons, New York.

[71] Vavon and Jakubowicz, *Bull. soc. chim.*, **53**, 581 (1933).

[72] v. Auwers, *Ann.*, **420**, 91 (1920); Skita, *Ber.* **53**, 1729 (1920).

[73] Grasshof, *Z. physiol. Chem.*, **225**, 197 (1934); Ruzicka, Brüngger, Eichenberger, and Meyer, *Helv. Chim. Acta*, **17**, 1407 (1934). Summary: Ruzicka, *ibid.*, **19E**, 9€ (1936).

* Proof that the spatial configuration of the C_3—OH group in dihydrocholesterol is the same as in coprosterol has been furnished by Reichstein and Lardon, *Helv. Chim. Acta*, **24**, 955 (1941). Catalytic hydrogenation of dehydroandrosterone acetate (p. 1503) in acetic acid with platinum as a catalyst gives the acetates of etio*allo*cholan-3(β)-ol-17-one (predominantly) and of etiocholan-3(β)-ol-17-one. The former is obtained also by chromic acid oxidation of dihydrocholesterol acetate, and the latter by oxidation of coprosterol acetate (*cf.* p. 1502).

of about 9 : 1.[74] When such an epimeric mixture is treated in alcoholic solution with the saponin digitonin (p. 1455), dihydrocholesterol and coprosterol form insoluble addition compounds and the epimeric addition

LXXXIII. Cholestanone

H(Pt) Neutral media / Acid media

LXXIX. Dihydrocholesterol (forms insoluble digitonide)

LXXXIV. Epidihydrocholesterol (forms soluble digitonide)

NaOR 180°C

90% / 10%

LXXXV. Coprostanone

H(Pt) Acid media / Neutral media

LXXXVI. Coprosterol (forms insoluble digitonide)

LXXXVII. Epicoprosterol (forms soluble digitonide)

NaOR 180° C.

10% / 90%
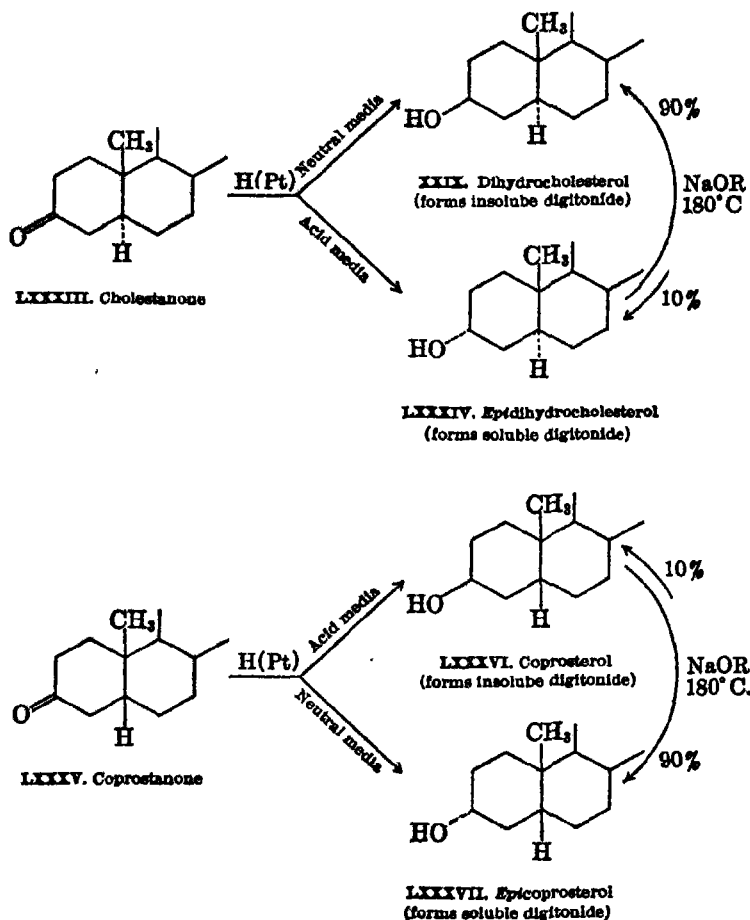
compounds remain in solution. If formation of an insoluble digitonide indicates a similarity of structure, it is difficult to reconcile this fact with the other reactions. Ruzicka,[75] from a consideration of models built with Stuart[76] atoms, offers the argument that the formation of an insoluble digitonide occurs when the hydroxyl group is unhindered and at

[74] Barnett, Heilbron, Jones, and Verrill, J. Chem. Soc., 1390 (1940). Earlier references are given in this article.

[75] Ruzicka, Furter, and Goldberg, Helv. Chim. Acta, 22, 498 (1938).

[76] Stuart, Z. physik. Chem., 27B, 350 (1934).

the end of a long axis. Because of the uncertainty of arguments based on mechanical models, and especially on Stuart models,[77] this contention is not wholly convincing.

From the study of numerous epimeric alcohols among the steroids and among model compounds, it is evident that transoid forms undergo dehydration less readily than the cisoid, and that esters of the transoid alcohols are hydrolyzed more easily than those of the cisoid.[75] When the four saturated sterols are examined by these reactions, dihydrocholesterol is dehydrated more easily than *epi*dihydrocholesterol, but there are no satisfactory data on the behavior of the coprosterols. The esters of dihydrocholesterol are hydrolyzed more rapidly than those of *epi*dihydrocholesterol, and the esters of *epi*coprosterol are hydrolyzed somewhat more rapidly than those of coprosterol.

Related both to ester formation and to digitonide formation is the behavior of the stereoisomers in the ease of glucoside formation. Miescher[78] concluded that glucoside formation occurs readily with dihydrocholesterol and coprosterol but does not occur with the epimers. This apparent analogy to digitonide formation has been discredited by Linstead,[79] who, using only dihydrocholesterol and *epi*hydrocholesterol, showed that glucoside formation is equally rapid with both epimers. The glucosides from *epi*dihydrocholesterol are more soluble and are more difficult to isolate, however, than those from dihydrocholesterol.

W. Stoll[80] has suggested another means of establishing an *epi* configuration. The *p*-toluenesulfonates of the normal (β) saturated sterols react slowly when boiled with methyl alcohol to form methyl ethers, but the *p*-toluenesulfonates of the epimers are converted into unsaturated compounds by the same treatment. It is difficult to evaluate this method because it has been applied to relatively few compounds.

Walden Inversion of the C_3—OH. When cholesterol (II) is treated with phosphorus pentachloride or thionyl chloride, the hydroxyl is replaced with chlorine. Subsequent regeneration of the hydroxyl by treatment of cholesteryl chloride with potassium acetate followed by saponification returns the original cholesterol. If, however, the cholesteryl chloride is catalytically hydrogenated and the chlorine then replaced with hydroxyl, *epi*dihydrocholesterol is obtained rather than dihydrocholesterol, and, obviously, at some stage of this transformation there is a Walden rearrangement about C_3.[81] The two chlorinating

[77] *Cf.* Marvel and Glass, *J. Am. Chem. Soc.*, **60**, 1051 (1938).

[78] Miescher and Fischer, *Helv. Chim. Acta*, **21**, 336 (1938).

[79] Linstead, *J. Am. Chem. Soc.*, **62**, 1766 (1940).

[80] Stoll, *Z. physiol. Chem.*, **246**, 1 (1937).

[81] Marker, *J. Am. Chem. Soc.*, **57**, 1755 (1935); Marker, Whitmore, and Kamm, *ibid.*, **57**, 2358 (1935). *Cf.* Bergmann, *Helv. Chim. Acta*, **20**, 590 (1937).

reagents behave differently with dihydrocholesterol and its epimer. Phosphorus pentachloride with dihydrocholesterol gives the so-called β-cholestyl chloride, m.p. 102°,[82] but thionyl chloride produces α-cholestyl chloride, m.p. 112°.[83] With epidihydrocholesterol the two reagents produce the α- and β-compounds, respectively. These transformations have been summarized by Marker[81] as follows:

Cholesterol $\underset{\text{KAc}}{\overset{\text{PCl}_5 \text{ or SOCl}_2}{\rightleftarrows}}$ Cholesteryl chloride ⟶

↓ H₂

Dihydrocholesterol $\underset{\text{KAc}}{\overset{\text{PCl}_5}{\rightleftarrows}}$ β-Cholestyl chloride

CrO₃ then H₂ ↓   SOCl₂

Epidihydrocholesterol $\underset{\text{KAc}}{\overset{\text{PCl}_5}{\rightleftarrows}}$ α-Cholestyl chloride ⟵
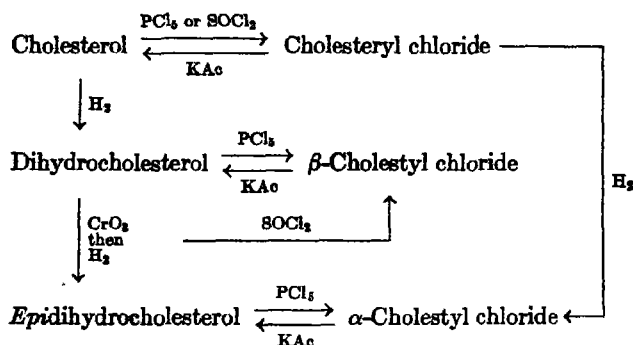
The uncertain nature of the changes that occur in the course of Walden inversion vitiate somewhat a proof that the C_3—OH of cholesterol is *cis* to the C_{10}—CH_3.[84] By suitable transformations cholesterol can be converted to 3-hydroxycholestan-6-one (LXXXVIII), a compound which appears to be spatially similar to cholesterol since both form insoluble digitonides. Treatment with phosphorus pentachloride converts this keto alcohol to β-3-chlorocholestan-6-one (LXXXIX), m.p. 180–181°. The isomeric α-3-chlorocholestan-6-one (XCII), m.p. 128–129°, may be obtained from cholesteryl chloride by nitration (at C_6) followed by reduction with zinc and acetic acid. On oxidation with nitric acid both chloroketones are opened in ring B to give chlorodicarboxylic acids, which, by treatment with alkali, are converted to the respective 3-hydroxydicarboxylic acids. The hydroxydicarboxylic acid (XC) from the β-3-chlorocholestanone forms a lactone, but the stereoisomer (XCIII) does not. Since, by the Alder-Stein[85] rule, lactone formation occurs only when hydroxyl and carboxyl groups are *cis* to each other, it follows that the C_3—OH of XC must be *cis* to the C_5—COOH and also to the C_{10}—CH_3. If the assumption is made that Walden inversion has not occurred in the transformation LXXXVIII–XC, or

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[81] Diels and Linn, *Ber.*, **41**, 548 (1908); Ruzicka, Goldberg, and Brünnger, *Helv. Chim Acta*, **17**, 1389 (1934).

[83] Ruzicka, Wirz, and Meyer, *Helv. Chim. Acta*, **18**, 998 (1935).

[84] Lettré, *Ber.*, **68**, 766 (1935).

[85] Alder and Stein, *Ann.*, **504**, 229 (1933).

that it has occurred twice, then the C_3—OH of cholesterol must be similarly *cis* to the C_{10}—CH_3. ●

LXXXVIII
3-Hydroxycholestan-6-one

$\xrightarrow{PCl_5}$

LXXXIX
β-3-Chlorocholestan-6-one

→

XC
(forms lactone)

XCI
6-Nitrocholesteryl chloride

$\xrightarrow{Zn}$

XCII
α-3-Cholorocholestan-6-one

→

XCIII
(does not form lactone)
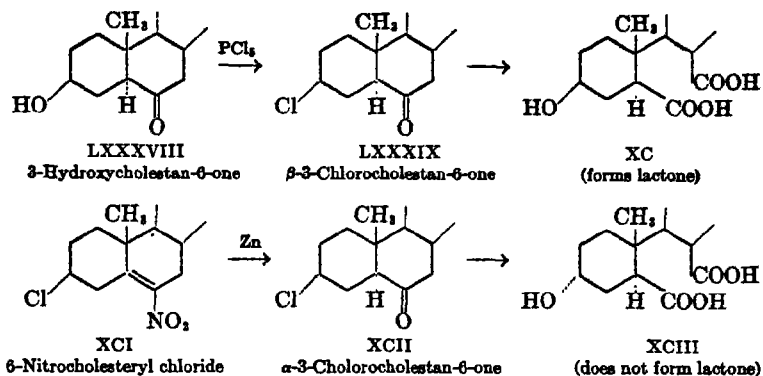
The spatial position of the C_3—OH group influences the physical properties of the steroids. Reindel and Niederländer [86] have compared the melting points of a large number of the saturated stereoisomers. In both the cholestane and the coprostane series, the member of the epimeric pair that gives an insoluble digitonide always has the lower melting point. Biochemically, the spatial configuration of the C_3—OH group (and of the C_{17}—OH group, also) is of great importance, although only in the cases of the cardiac principles and of the sex hormones is it possible to correlate configuration and physiological activity.

Other Ring Hydroxyl Groups. Where the hydroxyl groups are attached at positions other than C_3, the criteria for the determination of spatial configurations are not well developed except for the 17-hydroxy-steroids. These hydroxyl groups may occupy positions *cis* (β) or *trans* (α) to the neighboring C_{13}—CH_3 group, and may be examined as to ease of dehydration and of hydrolysis of esters. The 17(α)-hydroxy-steroids (*trans*) are dehydrated less easily than their epimers,[87] and the 17(α)-esters are saponified more readily than the corresponding β-deriv-atives.[75] In some cases formation of an insoluble digitonide occurs with the 17(α)-hydroxysteroid and not with the epimer; in others it takes place with both; and in still others with neither.

In the cases of the two bile acids, chenodesoxycholic (p. 1415) and cholic acids, there are hydroxyl groups at C_8 and C_7. Here it is possible by hypobromite oxidation to open rings A to 7-hydroxylithobilianic acids (*cf.* formula L, p. 1370) and examine for lactone formation. Since

[86] Reindel and Niederländer, *Ann.*, **522**, 218 (1936).

[87] Kägi and Miescher, *J. Soc. Chem. Ind.*, **57**, 276 (1938); *Helv. Chim. Acta*, **22**, 683 (1939); Woker and Antener, *ibid.*, **22**, 1309 (1939).

lactones are formed in both instances, the C_7—OH group must be *cis* to the C_5—COOH of lithobilianic acid, and *trans* to both the C_5—H and the C_{10}—CH$_3$.[34] By the convention used in this discussion, however, these C_7—OH groups are described as having the α-configuration.

Structure and Optical Rotation. Relatively little work has been done on the correlation of structure and optical rotation. From the molecular rotations of various pairs of compounds Callow and Young [1] have noted that epimerization of the C_3—OH group from the *cis* position with respect to the C_{10}—CH$_3$ group to a *trans* position is accompanied by a shift of rotation to the right. This increase of *d*-rotation is seen from the data given in the tables in the following discussion, where, for comparison, the specific rotations of isomers serve as well as the molecular rotations. Not enough cases of inversion of an hydroxyl group at C_4, C_5, or C_{17} were studied to permit conclusive generalizations to be made for these positions, but the introduction of a double bond into the molecule alters the *d*-rotation as follows: Compounds with unsaturation at Δ^1, Δ^5, and Δ^{22} show a marked decrease in *d*-rotation; at $\Delta^{8(14)}$, a small decrease; at Δ^7, an irregular effect; at Δ^{14}, a small increase; and at Δ^4, a marked increase. Reduction of a C_{17} carbonyl group to carbinol decreases the *d*-rotation slightly.

Lettré [88] has extended this work by studying the molecular rotations of neoergosterol (XCIV) and *epi*neoergosterol (XCV) and their derivatives. In these sterols there are no centers of asymmetry in ring B, and the rotation is due entirely to the effects of asymmetric carbon atoms, C_3, C_{13}, C_{14}, C_{17}, and the side chain. The asymmetric center at C_3 is so far removed from the other centers that the total rotation may be split up into two parts: part B, due to C_3; and part A, due to the rest of the molecule. On calculating the values for A and B in neoergosterol and *epi*neoergosterol, it is apparent that B (C_3) has a negative value in neoergosterol, but is positive in *epi*neoergosterol. The same relationship

Neoergosterol: $[\alpha]_D - 11°$; $[M]_D = -41.8° = A - B = 31.2° - 73°$

*Epi*neoergosterol: $[\alpha]_D + 27.4°$; $[M]_D = +104.2° = A + B = 31.2° + 73°$

applies to derivatives of the two sterols. Because of the sign of the rotation, neoergosterol may be regarded as a derivative of (—)-*ac*-tetrahydro-β-naphthol (XCVI) and *epi*neoergosterol of (+)-*ac*-tetrahydro-β-naphthol (XCVII) ($[M]_D = \pm 99.5$).[89] Since neoergosterol or *epi*neoergosterol can be correlated with the other compounds of the cyclopentano-perhydrophenanthrene group containing a C_3—OH group, all these compounds are related to the *ac*-tetrahydro-β-naphthols.

[88] Lettré, *Ber.*, **70**, 450 (1937).
[89] Pickard and Kenyon, *J. Chem. Soc.*, **101**, 1427 (1912).

From a consideration of the above and other results, Wallis [90] has concluded that rules, such as Hudson's carbohydrate rules (p. 1551), cannot be developed for the entire framework of the steroids, but that the effects of certain groupings may be evaluated. For example, the
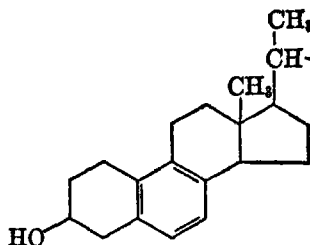
CH₃   C₂H₅ CH₃

$$CH_3 \quad\quad C_2H_5 \quad CH_3$$
$$CH-CH=CH-CH-CH$$
$$CH_3 \quad\quad\quad\quad CH_3$$

HO   HO

XCIV. Neoergosterol   XCV. *Epi*neoergosterol

HO   HO
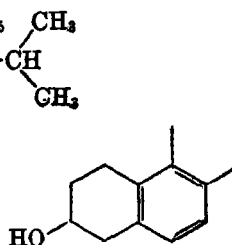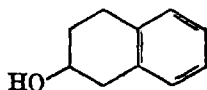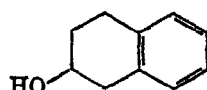
XCVI.(−) *ao*-Tetrahydro-β-naphthol   XCVII.(+) *ao*-Tetrahydro-β-naphthol

rotation due to a group at C_3 may be determined from known compounds and used in the study of new steroids.

THE STEROLS

The sterols may be defined as the saturated and unsaturated alcohols derived from cyclopentanoperhydrophenanthrene. By this definition some of the sex hormones and certain of the adrenal substances are sterols, but it is more convenient to discuss them separately. In nature the sterols are widely distributed, both free and combined as esters or glycosides (p. 1572). Esters of the sterols with fatty acids are common to both animal and plant life, but the glycosides, the so-called sterolins, occur only in plants. For classification, the sterols are divided on the basis of occurrence into zoösterols (animal sterols), phytosterols (plant sterols), and mycosterols (sterols of yeast and fungi).

The sterols are usually isolated in the form of well-crystallized compounds with a waxy feel from the unsaponifiable portion of fat extracts. Since more than one sterol is usually present in any natural product, separation is often difficult. The occurrence of mixed crystals and of molecular compounds sometimes makes purification of the free alcohol

[90] Bernstein, Kaufmann, and Wallis, *J. Org. Chem.*, **6**, 319 (1941); Bernstein, Wilson, Jr., and Wallis, *ibid.*, **7**, 103 (1942).

TABLE I

PRINCIPAL NATURAL AND DERIVED STEROLS *

Sterol †	Structure	Formula	$\|=$‡	Insoluble Digitonide	M.P. °C.	$[\alpha]_D$ (CHCl₃)	Source or Derivation
Zoösterols							
7-Dehydrocholesterol	3(β)-Hydroxy-$\Delta^{5,7}$-cholestadiene	C₂₇H₄₄O	2	+	142–143.5	−113.6°	Cholesterol
Allocholesterol	3(β)-Hydroxy-Δ^{4}-cholestene	C₂₇H₄₆O	1	+	132 c.	+43.7§	Cholestenone
Epiallocholesterol	3(α)-Hydroxy-Δ^{4}-cholestene	C₂₇H₄₆O	1	−	94 c.	+120.8§	Cholestenone
Cholesterol	3(β)-Hydroxy-Δ^{5}-cholestene	C₂₇H₄₆O	1	+	150–151	−39.5	All animal tissues, esp. brain and spinal cord; gallstones
Epicholesterol	3(α)-Hydroxy-Δ^{5}-cholestene	C₂₇H₄₆O	1	−	141	−35	Cholesteryl chloride
i-Cholesterol		C₂₇H₄₆O	0	−	74–75	+23.9	Cholesteryl p-toluenesulfonate
7-Hydroxycholesterol	3(β),7-Dihydroxy-Δ^{5}-cholestene	C₂₇H₄₆O₂	1		172	+94.2	Cholesterol
β-7-Hydroxycholesterol	3(β),7-Dihydroxy-Δ^{5}-cholestene	C₂₇H₄₆O₂	1		184–185	−86.4	Cholesterol
Dihydrocholesterol	3(β)-Hydroxycholestane	C₂₇H₄₈O	0	+	140–141	+28.8	Cholesterol, feces
Epidihydrocholesterol	3(α)-Hydroxycholestane	C₂₇H₄₈O	0	−	183–184	+33.9	Cholesterol
Coprosterol	3(β)-Hydroxycoprostane	C₂₇H₄₈O	0	+	100–101 c.	+28	Feces, cholesterol
Epicoprosterol	3(α)-Hydroxycoprostane	C₂₇H₄₈O	0	−	111 c.	+31	Coprostanone
Ostreasterol		C₂₉H₄₈O	2	+	142–143	−43.6	Oysters, clams
Phytosterols							
Brassicasterol	3(β)-Hydroxy-24-methyl-$\Delta^{5,22}$-cholestadiene	C₂₈H₄₆O	2	+	146	−61	Rapeseed oil
7-Dehydrostigmasterol	3(β)-Hydroxy-24-ethyl-$\Delta^{5,7,22}$-cholestatriene	C₂₉H₄₆O	3	+	154	−113.1	Stigmasterol

α-Spinasterol	3(β)-Hydroxy-24-ethyl-$\Delta^{8(14),22}$-cholestadiene	$C_{29}H_{48}O$	2	+	172 c.	− 3 7	Spinach, alfalfa
Fucosterol	3(β)-Hydroxy-24-ethyl-$\Delta^{5,8(14)}$-cholestadiene (?)	$C_{29}H_{48}O$	2	+	124	− 38 4	Bladder wrack
α₁-Sitosterol		$C_{29}H_{48}O$	2	+	166	− 1 7	Wheat germ oil
Stigmasterol	3(β)-Hydroxy-24-ethyl-$\Delta^{5,22}$-cholestadiene	$C_{29}H_{48}O$	2	+	169–170	− 51	Soy and calabar beans
α₂-Sitosterol		$C_{29}H_{48}O$	2	+	142–143	+ 5.2	Wheat germ oil
β-Sitosterol	22-Dihydrostigmasterol	$C_{29}H_{50}O$	1	+	136–137	− 34 2	"Tallöl," sarsaparilla root, cottonseed oil, cinchona bark
γ-Sitosterol	3(β)-Hydroxy-24-ethyl-cholestane	$C_{29}H_{50}O$	1	+	145–146	− 42	Chief sterol of plants
γ-Stostanol		$C_{29}H_{52}O$	0	+	144–145 c.	+ 27.8	γ-Sitosterol
Stigmastanol		$C_{29}H_{52}O$	0	+	137	+ 24 8	Stigmasterol
Epistigmastanol	3(α)-Hydroxy-24-ethyl-cholestane	$C_{29}H_{52}O$	0	−	200	+ 25	Stigmasterol
α₃-Sitosterol		$C_{30}H_{50}O$	1	+	156	+ 3.5	Wheat germ oil
Mycosterols							
Zymosterol	3(β)-Hydroxy-$\Delta^{8(14),24}$-cholestadiene	$C_{27}H_{44}O$	2	+	108–110	+ 47 3	Yeast
Ergosterol	3(β)-Hydroxy-24-methyl-$\Delta^{5,7,22}$-cholestatriene	$C_{28}H_{44}O$	3	+	163	−132	Ergot, yeast
22-Dihydroergosterol	3(β)-Hydroxy-24-methyl-$\Delta^{5,7}$-cholestadiene	$C_{28}H_{46}O$	2		152–153	−109	Ergosterol
Ergostanol	3(β)-Hydroxy-24-methylcholestane	$C_{28}H_{50}O$	0	+	150–151	0	Ergosterol

* Data selected largely from the compilation of Sobotka, "The Chemistry of the Sterids," Williams and Wilkins, Baltimore (1938).

† In the older literature, the following terms have been used: β-cholestanol for dihydrocholesterol; γ-cholestanol for a molecular compound of dihydrocholesterol and epicoprosterol; δ-cholestanol for epicoprosterol; and ε-cholestanol for epidihydrocholesterol.

‡ Double bonds.

§ In benzene.

by crystallization impracticable. In this case, advantage is taken of the differential solubilities of derivatives such as the dibromides of unsaturated sterols and the digitonides, or the mixture is resolved by chromatographic analysis.[91]

Because of the difficulty of obtaining pure compounds, many of the analyses reported in the older literature are erroneous. Even though a pure compound is available for analysis, the results may be hard to interpret. For this reason, analyses are based frequently on the acetate and on the dinitrobenzoate rather than on the free sterol.[92]

The physical properties of a number of the more important sterols are given in Table I. In this table the compounds are named as derivatives of the stereoisomeric hydrocarbons, cholestane and coprostane (p. 1367). With the unsaturated sterols, particularly those with unsaturation at C_5, there is a problem of reference compound. It seems best to refer them all to cholestane, rather than to relate some to this hydrocarbon and others to coprostane. The sterols derived from the two hydrocarbons pregnane (p. 1489) and urane (p. 1496) do not fit in this general classification and are treated separately.

General Reactions of the Sterols

The reactions of the sterols may be discussed in relation to type formula I. As this structure shows, there is an hydroxyl group at C_3, and in many of the natural sterols there is unsaturation at C_5. The side
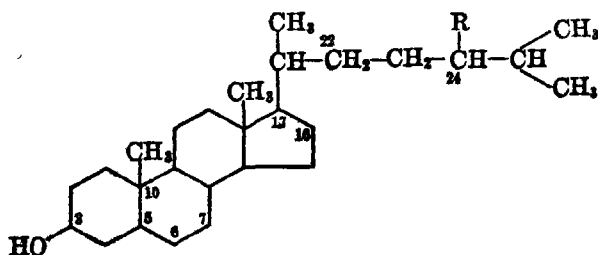
I. Ring system of the sterols
Rings A/B: *cis* or *trans*. The nucleus is usually unsaturated at C_5: C_6; it is sometimes unsaturated at C_7. The side chain may be unsaturated at C_{22} or at C_{24}.

chain attached at C_{17} is generally isoöctyl or substituted isoöctyl. In a few of the sterols there is unsaturation in the side chain at C_{22} or C_{24}, and in a number of them the side chain is substituted with a methyl or

[91] *Cf., inter al.,* Ladenburg, Fernholz, and Wallis, *J. Org. Chem.,* **3**, 294 (1938); Brockmann, *Angew. Chem.,* **53**, 384 (1940); Sobel and Spoerri, *J. Am. Chem. Soc.,* **64**, 361 (1942).

[92] Sandqvist and Gorton, *Ber.,* **63**, 1935 (1930); Sandqvist and Bengtsson, *Ber.,* **64**, 2167 (1931); Windaus, Werder, and Gschaider, *Ber.,* **65**, 1006 (1932).

an ethyl group at C_{24}. These characteristic features of the sterol molecule are doubtless of biogenetic significance.

The C_3—OH. In addition to the reactions of a secondary hydroxyl group, the C_3—OH undergoes a number of changes that are conditioned by the structure of the molecule and by the nature of the reagents used. The Walden inversion that occurs when the saturated sterols are treated with chlorinating agents and regenerated has been discussed (p. 1375). An unusual kind of rearrangement that involves more fundamental changes is known for the Δ^5-unsaturated sterols.

Cholesteryl p-toluenesulfonate and methanol react smoothly to give two different cholesteryl methyl ethers.[93] With methanol alone, the expected l-ether, m.p. 84°, $[\alpha]_D$-42°, is obtained,[94] but with methanol and potassium acetate a d-ether, m.p. 79°, $[\alpha]_D + 51.8°$, results. Similarly, from cholesteryl chloride the l-ether is formed by treatment with methanol alone, and the d-ether by treatment with methanol and potassium acetate.[95] Analogous acetates are obtained if the p-toluenesulfonate is treated with acetic anhydride in the absence, or in the presence, of potassium acetate. On hydrolysis of the d-derivatives, the compound known as i-cholesterol (II) is obtained.[96] The structure assigned to this sterol is based on the following arguments: The hydroxyl group is attached at C_6 for, on chromic acid oxidation of i-cholesterol, a ketone (III) is formed, which on treatment with hydrogen chloride in acetic acid is converted to α-3-chlorocholestan-6-one (p. 1377). The C_5 double bond of cholesterol is no longer present in i-cholesterol since the latter compound, and its derivatives, are not acted upon by perbenzoic acid or ozone, and do not brominate readily. When bromination does occur,
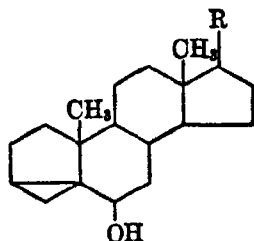
II. i-Cholesterol   III
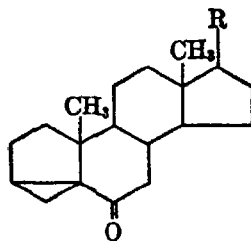
[93] Stoll, *Z. physiol. Chem.*, **207**, 147 (1932); **246**, 6 (1937).

[94] Diels and Blumberg, *Ber.*, **44**, 2847 (1911); Bills and MacDonald, *J. Biol. Chem.*, **72**, 1 (1927).

[95] Wagner-Jauregg and Werner, *Z. physiol. Chem.*, **213**, 119 (1932).

[96] Wallis, Fernholz, and Gephart, *J. Am. Chem. Soc.*, **59**, 137 (1937); Ford, Chakravorty, and Wallis, *ibid.*, **60**, 413 (1938); Beynon, Heilbron, and Spring, *J. Chem. Soc.*, 907 (1938). *Cf. idem, ibid.*, 907 (1936); 406, 1459 (1937); Butenandt and Grosse, *Ber.*, **70**, 1446 (1937).

the product is not a 5,6-dibromo-addition derivative but a 3,5,6-tri-bromo-substitution-addition compound. *i*-Cholesterol can be rearranged to cholesterol by various procedures, such as heating with zinc acetate and acetic acid, and it can be hydrogenated to dihydrocholesterol with platinum as a catalyst. The assumption of the cyclopropane ring is compatible with these reactions, but there is no direct proof of such a structure. This ring, if present, is much more stable in the dicarboxylic acids formed by opening ring B through alkaline oxidation with permanganate than in the parent compound.[97]

From the study of other sterols [98] and of a number of compounds related to the male sex hormones,[99] it is evident that the formation of *i*-steroids from normal steroids with an hydroxyl group at C_3 and a double bond at C_5 is one of the general reactions of the group.

The C_{17} Side Chain. Except in the members of the pregnane and the urane series (p. 1487), the C_{17} side chain of the natural sterols is an isoöctyl or substituted isoöctyl group. Where there is unsaturation in this side chain, it is usually at C_{22}. In determining the structure of such a side chain the position of the double bond is placed by examining the volatile aldehyde produced by the action of ozone. Thus, ethyliso-propylacetaldehyde is obtained from stigmasterol, and methylisopropyl-acetaldehyde from ergosterol and its irradiation products.[100]

Chromic acid oxidation of the acetylated saturated sterols, or of the sterol hydrocarbons, cleaves the side chain at the linkages with tertiary carbon atoms.* The composition of the mixture obtained by such an

[97] Ladenburg, Chakravorty, and Wallis, *J. Am. Chem. Soc.*, **61**, 3483 (1939).

[98] Fernholz and Ruigh, *ibid.*, **62**, 3346 (1940).

[99] Butenandt and Grosse, *Ber.*, **70**, 1446 (1937).

[100] Reindel and Kipphan, *Ann.*, **493**, 181 (1932); Guiteras, Nakamiya, and Inhoffen, *Ann.*, **494**, 116 (1932).

* The variety of compounds that can be obtained by chromic acid oxidation of a sterol side chain is illustrated by the products that have been isolated from the oxidation of cholesterol (as acetate dibromide) under various conditions. In addition to $3(\beta)$-hydroxy-Δ^5-cholenic acid (p. 1415), Δ^5-pregnenolone (p. 1488), and dehydroandrosterone (p. 1502), the following have been isolated: $3(\beta)$-hydroxy-Δ^5-norcholesten-25-one (A) [by Ruzicka and Fischer, *Helv. Chim. Acta*, **20**, 1291 (1937)], an hydroxy lactone formulated as B by Miescher and Fischer, *ibid.*, **27**, 155 (1939), and an unsaturated ketonic alcohol (C) by Köster and Logemann, *Ber.*, **73**, 298 (1940).
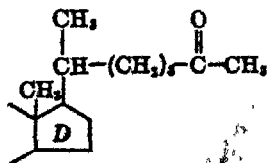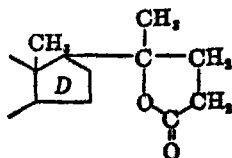
A. $3(\beta)$-Hydroxy-Δ^5-norcholesten-25-one   B. Hydroxylactone   C. Unsaturated alcohol
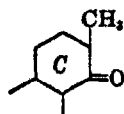
oxidation is dependent on the temperature at which the oxidation is carried out but can be resolved into volatile and non-volatile ketones, acidic constituents, etc. The volatile ketones aid in determining the structure of the side chain. For example, from dihydrocholesterol, isohexyl methyl ketone was isolated early in the course of the study of the structure of cholesterol.[101] The non-volatile ketones, i.e., ketones with carbonyl at C_{20} or C_{17}, are useful intermediates in the preparation of the sex hormones.[102] By oxidative cleavage at C_{24}—C_{25} or, where C_{24} is substituted by alkyl, at C_{23}—C_{24}, bile acid derivatives are obtained that have been of value in determining the stereochemical relationships of the nucleus and of the C_3—OH. As an illustration, 3(β)-hydroxy*allo*cholanic acid is formed as one of the products of the oxidation of acetyldihydrocholesterol, and its isolation served as one of the ways of establishing the spatial relation of the hydroxyl group and of rings A/B.[103]

The Nuclear Unsaturation. Nearly all the natural sterols are unsaturated in the 5,6-position. The method of determining the position of this double bond has been illustrated in the discussion of the structure of cholesterol (p. 1354) and has been tested on a sufficiently large number of sterols to establish its general applicability. The double bond in the 5,6-position is more reactive, apparently, than a double bond placed elsewhere in the molecule, including the side chain.

The methylene groups adjacent to an ethylenic bond at $C_5 : C_6$ are easily oxidized to carbinols or carbonyls. If the mild oxidizing agent selenium dioxide[104] is used in suitable solvents (acetic acid, etc., but not alcohol), cholesterol yields Δ^5-cholestene-3,4-diol, m.p. 176°, $[\alpha]_D$ −60°. With cholesteryl acetate nearly equal amounts of the 3,4-diol and of Δ^4-cholestene-3,6-diol, m.p. 257°, $[\alpha]_D$ + 6°, are formed. The reaction is shown schematically below:

Δ^5-3,4-diol Δ^4-3,6-diol
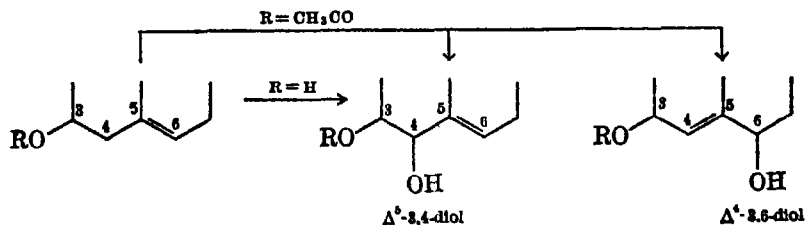
[101] Windaus and Resau, *Ber.*, **46**, 1246 (1913); Windaus and Neukirchen, *Ber.*, **52**, 1915 (1919); Windaus, *Z. physiol. Chem.*, **145**, 177 (1925).

[102] *Inter al.*, Ruzicka, Goldberg, and Brüngger, *Helv. Chim. Acta*, **17**, 1389 (1934); Ruzicka and Fischer, *ibid.*, **20**, 1291 (1937); Miescher and Fischer, *ibid.*, **22**, 155 (1939).

[103] Fernholz and Chakravorty, *Ber.*, **67**, 2021 (1934).

[104] Rosenheim and Starling, *J. Chem. Soc.*, 377 (1937); Butenandt and Hausmann, *Ber.*, **70**, 1154 (1937); Marker, Kamm, and Wittle, *J. Am. Chem. Soc.*, **60**, 1071 (1938); Marker and Rohrmann, *ibid.*, **60**, 1073 (1938).

The use of stronger oxidizing agents leads to the addition of hydroxyl groups to the ethylene linkage. The course of the reaction is somewhat dependent on the medium. From cholesterol, for example, employment of alkaline permanganate leads directly to one of the isomeric cholestane-triols (p. 1355), while acid permanganate apparently gives a mixture of isomeric oxides which are then converted to triols.[106] In the latter case, an excess of oxidizing agent converts the triols to 3,6-diketo-5-hydroxy derivatives.

If the esters of the unsaturated sterols are oxidized with chromic acid or permanganate in acetic acid under mild conditions,* the action is principally at C_7.[104] Chromic acid at 40° converts sterol esters to 7-ketosterols (IV) which, on reduction with aluminum isopropoxide, give mixtures of isomeric 7-hydroxysterols (V) in which the low-melting α-isomer predominates. The β-stereoisomer (β-7-hydroxy) may be pro-
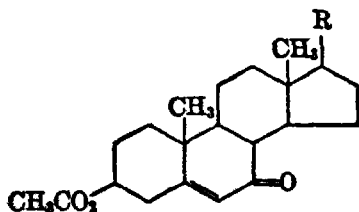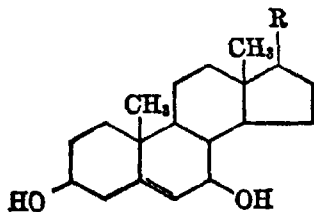
IV. 7-Ketosteryl acetate   V. 7-Hydroxysterol

duced directly by permanganate oxidation of the acid phthalate ester of the sterol.[107] By benzoylation and pyrolysis the newly introduced hydroxyl groups are split out to give dehydrosterols with the conjugated system $C_5 : C_6 \cdot C_7 : C_8$. As a by-product of the pyrolysis of the dibenzoate of 7-hydroxycholesterol, a small amount of isodehydrocholesterol is formed. This isomer of 7-dehydrocholesterol probably contains the conjugated system $C_6 : C_7 \cdot C_8 : C_9$.[108] Treatment of either dehydro-

[105] Marker and Rohrmann, J. Am. Chem. Soc., 62, 516 (1940); Ehrenstein and Decker, J. Org. Chem., 5, 544 (1940).

* Colloidal solutions of cholesterol are converted by aeration with molecular oxygen to 7(α)-hydroxycholesterol (ca. 30 per cent yield) and 7-ketocholesterol [Wintersteiner and Bergström, J. Biol. Chem., 137, 785 (1941)]. This shows the susceptibility of position 7 to attack by oxygen and suggests a path by which cholesterol may be converted in biological systems to 7-dehydrocholesterol and vitamin D₃.

[106] Mauthner and Suida, Monatsh., 17, 496 (1896); Windaus, Ber., 53, 488 (1902); Windaus, Lettré, and Schenck, Ann., 520, 98 (1935); Wunderlich, Z. physiol. Chem., 241, 116 (1936); Linsert, ibid., 241, 125 (1936); Marker, Kamm, Fleming, Popkin, and Wittle, J. Am. Chem. Soc., 59, 619 (1937); Windaus and Schenck, U. S. pat., 2,098,984; Marker, U. S. pat., 2,177,355.

[107] Barr, Heilbron, Parry, and Spring, J. Chem. Soc., 1437 (1936).

[108] Windaus, Linsert, and Eckhardt, Ann., 534, 22 (1938).

sterol with hydrogen chloride gives the same product, probably with distribution of the conjugated system between rings B and D.[108]

When a dehydrosterol (VI) is reduced with sodium and alcohol, only one of the double bonds is saturated. The resulting γ-stenol (VII), in

VI. 7-Dehydrosterol

VII. γ-Stenol

VIII. α-Stenol

IX. β-Stenol

X. Pinacol
(bidehydrosterol)

XI. Norsterol
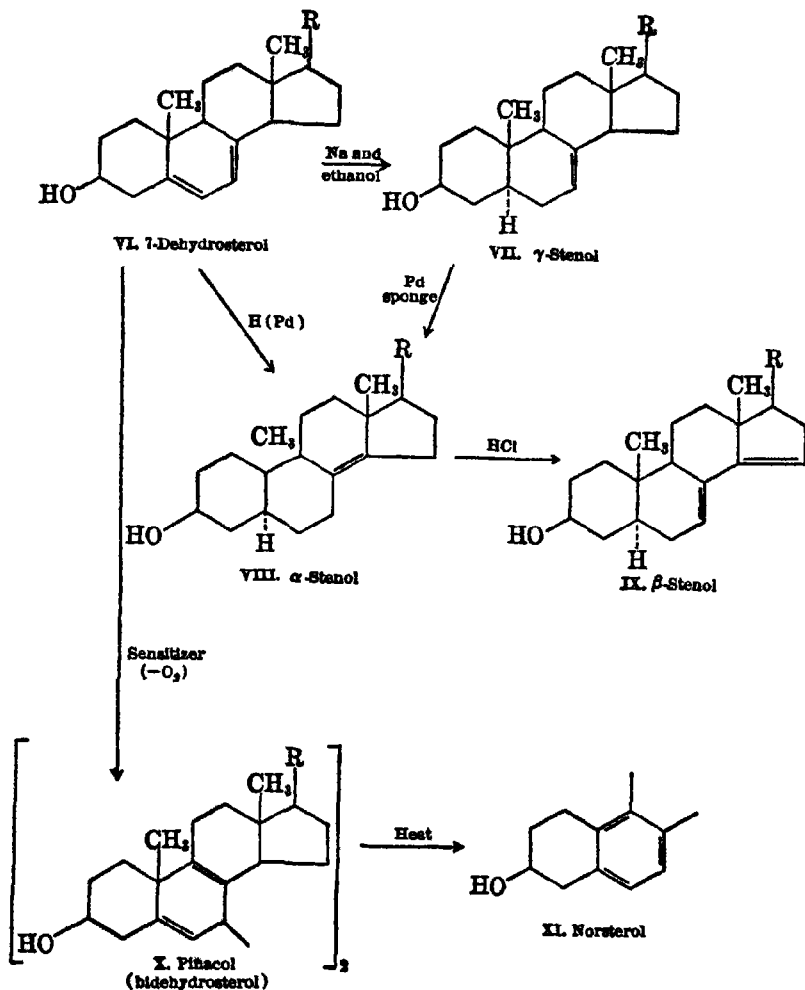
which the double bond is probably at $C_7 : C_8$, is rearranged into an α-stenol (VIII) by shaking with palladium sponge. The double bond of the α-stenol is resistant to catalytic hydrogenation; it is probably located at $C_8 : C_{14}$. The α-stenol may be produced directly with the uptake of one mole of hydrogen by catalytic reduction of the dehydrosterol in the

presence of palladium. Although an α-stenol cannot be hydrogenated, it can be rearranged by treatment with hydrogen chloride in chloroform solution to a β-stenol (IX), which takes up hydrogen readily to give the completely saturated sterol.[109]

If alcoholic solutions of dehydrosterols are irradiated in the presence of oxygen and of a sensitizer like eosin, transannular peroxides are formed through 1,4-addition of oxygen to the conjugated system in ring B.[110] Like other peroxides of this class, the products are unusually stable. Transannular peroxides are formed also with conjugated systems in other portions of the nucleus, but the products are not as stable as those from the dehydrosterols (cf. 2,5-peroxido-Δ^3-cholestene, p. 1395). Irradiation of dehydrosterols with visible light in the absence of oxygen but with a sensitizer produces "pinacols."[111] Since these are formed by the loss of hydrogen from two molecules of the dehydrosterol, Jacobsen[111] has suggested that they are better described as "bi-dehydrosterols." The provisional formula X proposed by Inhoffen[111] for the pinacols from 7-dehydrosterols with a 7,7'-linkage is probably representative of all pinacols formed from steroids with a conjugated system in ring B. Pinacols are obtained from steroids with conjugated systems in other portions of the ring structure, but their formulation is less secure.[112] The pinacols from the 7-dehydrosterols are unstable when heated above their melting points, or when boiled in acetic anhydride, and are converted to the semi-benzenoid norsterols (XI) by the loss of methane from C_{10}—CH_3 and C_9—H.

The dehydrosterols have a characteristic absorption spectrum in the ultra-violet within the range 260–300 mμ, with maxima at ca. 270 mμ, 280 mμ, and 295 mμ.* The absorption is due entirely to the conjugated system in ring B, and the absolute position of the bands is unaffected by the surrounding structure.[113] The intensity of the absorption, however, varies as the surrounding structure is modified.

The Sterol Ketones. By cold oxidation of the saturated sterols, or by dehydrogenation of the unsaturated sterols using Oppenauer's method,[114] the sterol ketones are obtained in excellent yield. The

[109] Achtermann, Z. physiol. Chem., **225**, 141 (1934); Laucht, ibid., **237**, 236 (1935); Dimroth and Trautmann, Ber., **69**, 669 (1936).

[110] Review: Bergmann and McLean, Chem. Rev., **28**, 367 (1941).

[111] Windaus and Borgeaud, Ann., **460**, 235 (1928); Inhoffen, Naturwissenschaften, **25**, 125 (1937); cf. Jacobsen and Nawrocki, J. Am. Chem. Soc., **62**, 2612 (1940), for bibliography; and, also, Windaus and Zühlsdorff, Ann., **536**, 204 (1938).

[112] Cf. Butenandt and Poschmann, Ber., **73**, 893 (1940).

* For typical curve (ergosterol) see Morton, "The Application of Absorption Spectra to the Study of Vitamins and Hormones," Hilger, London (1935), p. 8.

[113] Dimroth and Trautmann, Ber., **69**, 669 (1936).

[114] Cf. Bersin, Angew. Chem., **53**, 266 (1940), for review article.

3-ketones are the most accessible and are extremely valuable as intermediates for further transformations. The reactions of these 3-ketones are conditioned somewhat by the spatial arrangement of the molecule.

The saturated ketones with the *allo* or *trans* configuration of rings A/B, such as cholestan-3-one (XII), are substituted at C_2 by bromina-

tion [115] or sulfonation. [116] With continued bromination, a second bromine atom is introduced at C_4. [117] On dehydrobrominating the 2-monobromoketones with collidine (2,4,6-trimethylpyridine) the expected Δ^1-ketone is obtained together with some of the parent saturated ketone. [118] For example, 2-bromocholestan-3-one (XIII) on treatment with collidine gives a mixture of Δ^1-cholesten-3-one (XIV) and cholestan-3-one. [119] Treatment of the 2-bromoketone with pyridine, or with pyridine derivatives which are unsubstituted in the 4-position, gives rise first to pyridinium compounds [118] which are evidently then rearranged and finally decomposed to Δ^4-ketones. [120] Dehydrobromination of the 2-bromoketones with potassium acetate in acetic acid leads to the formation of compounds of uncertain structure which Butenandt [118] has designated as h-Δ^1-ketones.
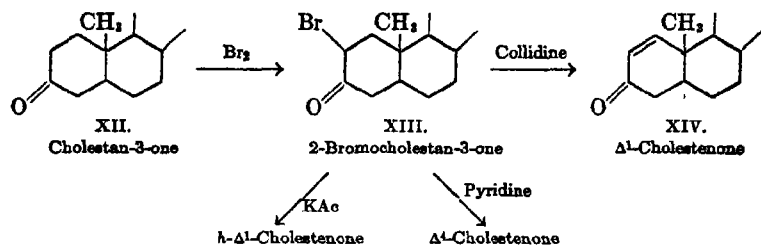
The 3-ketones derived from the coprostane type brominate at C_4. [117] Two atoms of bromine may enter to form first a monobromo- and then a dibromoketone. Sulfonation, on the other hand, takes place both at C_2 and at C_4. [121] Debromination of the monobromo derivatives proceeds in the normal manner to give Δ^4-ketones.

These differences in the behavior of the two types have been utilized

[115] Butenandt and Mamoli, *Ber.*, **68**, 1850, 1854 (1935); Butenandt and Wolff, *Ber.*, **68**, 2091 (1935).

[116] Windaus and Kuhr, *Ann.*, **532**, 52 (1937).

[117] Butenandt and Schmidt-Thomé, *Ber.*, **67**, 1901 (1934); Butenandt, Schramm, Wolff, and Kudzus, *Ber.*, **69**, 2779 (1936); Ruzicka, Bosshard, Fischer, and Wirz, *Helv. Chim. Acta*, **19**, 1147 (1936); Inhoffen, *Ber.*, **70**, 1695 (1937).

[118] Butenandt, Mamoli, Dannenberg, Masch, and Poland, *Ber.*, **72**, 1617 (1939); Inhoffen, Zühlsdorff, and Huang-Minlon, *Ber.*, **73**, 451 (1940).

[119] Jacobsen, *J. Am. Chem. Soc.*, **62**, 1620 (1940).

[120] Ruzicka, Plattner, and Aeschbacher, *Helv. Chim. Acta*, **21**, 866 (1938).

[121] Windaus and Mielke, *Ann.*, **536**, 116 (1938).

for structural determinations. Thus, the ketone obtained by oxidizing dibromocholesterol brominates at C_4, and since this parallels the behavior of coprostan-3-one, it is reasoned that the C_5—Br of dibromocholesterol is *cis* to the C_{10}—CH_3.[122] The product obtained by the addition of hydrogen halide to a C_5 double bond is largely made up of the isomer with the 5-halogen in a *cis* position to the C_{10}—CH_3, but the other isomeric and epimeric modifications are formed also in small amounts.[123]

The 3-ketones with unsaturation in conjugation with the carbonyl group present a more complicated situation since both substitution and addition may take place. The entrance of bromine is conditioned by the presence of acid, for no bromination occurs in the presence of potassium acetate. Substitution takes place by addition to an enolic form, and the entering halogen atoms form unstable intermediates which lose hydrogen bromide. The net effect is bromination at an unexpected position. This is illustrated in the bromination of Δ^4-cholestenone[124] where the final product is 4,6-dibromo-Δ^4-cholestenone (XVIa) or its enol (XVI). The course of the reaction (XV–XIX) has been studied by brominating the enolacetate in ether-acetic acid.

Reduction of the ring carbonyl to hydroxyl groups is easily accomplished by the use of aluminum isoalkoxides according to the Meerwein-Ponndorf procedure.[114] With the saturated steroid ketones catalytic reduction is equally satisfactory. Epimeric mixtures are usually obtained in both cases, but they are readily separated by the use of digitonin. Reduction of the carbonyl to a methylene group by the Wolff-Kishner method proceeds normally for all save the 3-ketones.[125] Here dissociation of the hydrazone or semicarbazone evidently interferes with the normal reduction, and the product is a mixture of epimeric 3-carbinols. By the addition of excess hydrazine, for example, Wintersteiner has shown that the dissociation may be reversed to give normal reduction.

The Color Reactions of the Sterols. The sterols give a number of color reactions which are useful for preliminary identification and for quantitative analysis, but which are not specific for the sterols. Since sulfuric and other strong acids are used in the tests, the colored substances are probably halochromic salts. The *Salkowski reaction*[126] is carried out by shaking a chloroform solution of the sterol with an equal

[122] Butenandt and Schramm, *Ber.*, **69**, 2289 (1936).

[123] Decombe and Rabinowitsch, *Bull. soc. chim.*, [5] **6**, 1510 (1939); de Fazi and Pirrone, *Gazz. chim. ital.*, **70**, 18 (1940).

[124] Inhoffen, *Ber.*, **69**, 2141 (1936).

[125] Marker and Lawson, *J. Am. Chem. Soc.*, **61**, 852 (1939); Dutcher and Wintersteiner, *ibid.*, **61**, 1992 (1939); Marker, Turner, and Uhlshafer, *ibid.*, **62**, 3009 (1940).

[126] Salkowski, *Z. physiol. Chem.*, **57**, 523 (1908).

volume of concentrated sulfuric acid. The *Liebermann-Burchard reaction* [127] is identical with the Salkowski reaction save that a few drops of acetic anhydride are added to the mixture. The *Rosenheim test* [128] differs from the others in that the free sterol or a chloroform solution of the sterol is treated with 90 per cent trichloroacetic acid. Color production
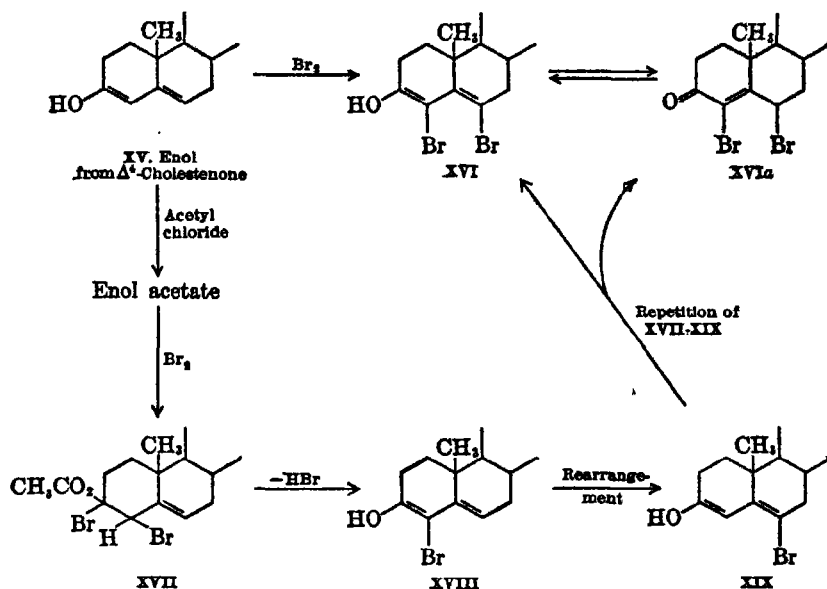
indicates a system containing, or potentially containing, a conjugated system of carbon atoms. [129]

By underlayering with sulfuric acid an alcoholic solution of a sterol without [130] or with an aldehyde, such as benzaldehyde [131] or furfuraldehyde, [132] characteristic colors are produced at the interface. The rate at which these color bands are produced, as well as their hue and intensity, may be related to the steric configuration of the hydroxyl group.

Molecular Compounds. One of the characteristics of the sterols is their ability to form molecular compounds and mixed crystals with other sterols. By means of melting-point diagrams, Lettré [133] has

[127] Liebermann, *Ber.*, **18**, 1803 (1885); Burchard, *Chem. Zentr.*, **61** (I), 25 (1890); Schoenheimer and Dam, *Z. physiol. Chem.*, **215**, 59 (1933).

[128] Rosenheim, *Biochem. J.*, **23**, 47 (1929); Rosenheim and Callow, *ibid.*, **25**, 74 (1931).

[129] Heilbron and Spring, *J. Chem. Soc.*, 2664 (1930); Schoenheimer and Evans, Jr., *J. Biol. Chem.*, **114**, 567 (1936).

[130] Woker and Antener, *Helv. Chim. Acta*, **22**, 1309 (1939).

[131] Scherrer, *ibid.*, **22**, 1329 (1939).

[132] Woker and Antener, *ibid.*, **22**, 511 (1939).

[133] Lettré, *Ann.*, **495**, 41 (1932).

studied the relationship between these tendencies and structure. If cholestanol and coprostanol (coprosterol) and their epimers are used as examples representing all the possible arrangements with respect to the C_{10}—CH_3 group at the points of asymmetry, C_3 and C_5, the following relationships obtain:

	C_3—OH	C_5—H			C_3—OH	C_5—H
Cholestanol	*cis*	*trans*		Coprostanol	*cis*	*cis*
Epicholestanol	*trans*	*trans*		Epicoprostanol	*trans*	*cis*

Compound formation takes place between cholestanol and *epi*coprostanol, or between coprostanol and *epi*cholestanol. Molecular compounds are formed, therefore, by pairs which are structurally dissimilar with respect to both the C_3—OH and the C_5—H. The spatial position of the hydroxyl group appears to be the determining factor, since masking the hydroxyl by acetylation destroys the capacity for compound formation. One of the components of a pair may be replaced by a structurally similar compound; for example, ergostanol, $3(\beta)$-hydroxy-24-methylcholestane, forms a molecular compound with *epi*coprostanol.

Mixed crystals are formed by sterols and 5,6-dihydrosterols, as with the pairs cholesterol-cholestanol and ergosterol-dihydroergosterol. Here, too, the hydroxyl group plays a part, but a minor one, for acetylation does not interfere with mixed crystal formation, whereas replacement of the hydroxyl by hydrogen does.

The Zoösterols

At one time it was thought that all the sterols associated with the animal kingdom had the same carbon content as cholesterol. One of the facts supporting this view was the isolation in 1872 of "isocholesterol" from wool fat.[134] Since that time this "sterol" has been resolved into two compounds: agnosterol,[135] $C_{30}H_{48}O$; and lanosterol,[136] $C_{30}H_{50}O$. Apparently neither of these substances is a sterol.[137] The isolation from animal tissues and excreta of a number of sterols of higher and lower carbon content than cholesterol has definitely disposed of the older belief.

Cholesterol and *Epi*cholesterol. Cholesterol, the principal animal sterol, is found in all tissues in amounts ranging from a few hundredths to 4–5 per cent.[138] Although cholesterol is doubtless of great importance to animal life, no specific function can be assigned to it. Generally it is

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[134] Schulze, *Ber.*, **5**, 1075 (1872); **6**, 251 (1873); **31**, 1200 (1898).

[135] Windaus and Tschesche, *Z. physiol. Chem.*, **190**, 51 (1930).

[136] Dorée and Garratt, *J. Soc. Chem. Ind.*, **52**, 141T, 355T (1933).

[137] Schulze, *Z. physiol. Chem.*, **238**, 35 (1936).

[138] *Cf.* Lettré and Inhoffen, "Über Sterine, Gallensäuren und verwandte Naturstoffe," Enke, Stuttgart (1936), p. 98.

regarded as an essential constituent of cells, but its synthesis and metabolism in the body are obscure.

*Epi*cholesterol, the epimer of cholesterol, is not found in nature but is obtained from cholesterol by synthetic methods. When 5,6-dibromocholestan-3-one is debrominated with zinc and acetic acid, Δ^4-cholestenone, m.p. 80°, $[\alpha]_D + 88.8°$, is obtained (p. 1357). If the debromination is carried on in weakly acidic alcohol, a double bond is produced without rearrangement, and Δ^5-cholestenone, m.p. 127°, $[\alpha]_D - 4.2°$, is obtained.[139] The Δ^5-ketone is readily rearranged into the Δ^4-ketone, but on catalytic reduction with Raney nickel, it is converted to a mixture of cholesterol and *epi*cholesterol.[140] The mixture is resolved by precipitating cholesterol with digitonin and extracting the *epi*cholesterol which does not form an insoluble digitonide. On a large scale, *epi*cholesterol can be separated from cholesterol by crystallizing first the acetates and then the benzoates from ethyl alcohol.

Marker[141] has employed cholesteryl chloride (p. 1376) as an intermediate for the formation of *epi*cholesterol. On oxygenating the Grignard from cholesteryl chloride and hydrolyzing, a mixture of cholesterol and *epi*cholesterol is obtained. By a second procedure, the chloride is oxidized with chromic acid in acetic acid at 55° to 7-ketocholesteryl chloride. On treatment with potassium acetate, an acetoxy group in *epi* configuration is substituted for the chlorine and the synthesis is completed by Wolff-Kishner reduction.

The sterol mixture present in the feces of man and of other Carnivora is largely coprosterol with small amounts of dihydrocholesterol and traces of cholesterol.[142] Since the bile contains cholesterol and some dihydrocholesterol,[143] there must be a biochemical mechanism to account for the stereoisomer coprosterol [3(β)-hydroxycoprostane]. Schoenheimer[144] has suggested that Δ^4-cholestenone is the intermediary substance in the formation of coprosterol.

Allocholesterol. In anhydrous media cholesterol adds hydrogen chloride to form the so-called cholesteryl chloride (C_5—Cl). Treatment of the addition product with anhydrous potassium acetate gives a mixture of allocholesterol (XX)* and cholesterol.† Because of the diffi-

[139] Butenandt and Schmidt-Thomé, *Ber.*, **69**, 882 (1936).

[140] Ruzicka and Goldberg, *Helv. Chim. Acta*, **19**, 1407 (1936).

[141] Marker, Oakwood, and Crooks, *J. Am. Chem. Soc.*, **58**, 481 (1936); Marker, Kamm, Oakwood, and Laucius, *ibid.*, **58**, 1948 (1936); Marker, U. S. pat., 2,177,355.

[142] Schoenheimer and co-workers, *Z. physiol. Chem.*, **192**, 73 (1930).

[143] Schoenheimer and Hrdina, *ibid.*, **212**, 161 (1932).

[144] Schoenheimer, Rittenberg, and Graff, *J. Biol. Chem.*, **111**, 183 (1935); *cf.* Rosenheim and Webster, *Nature*, **136**, 474 (1935).

* In this compound the prefix "allo" does not indicate a *trans* relationship of rings A/B.

† Originally the mixture itself was thought to be allocholesterol. *Cf.* Windaus, *Ann.*, **447**, 233 (1926); **433**, 101 (1927).

culty of resolving this mixture, allocholesterol is more easily obtained by catalytic reduction of Δ^4-cholesten-3-one.[145] The product of the reduction is a molecular addition compound, m.p. 141°, of allocholesterol and *epi*allocholesterol which is easily resolved through the insoluble digitonide of the former. Both allocholesterols are easily dehydrated to $\Delta^3,^5$-cholestadiene.
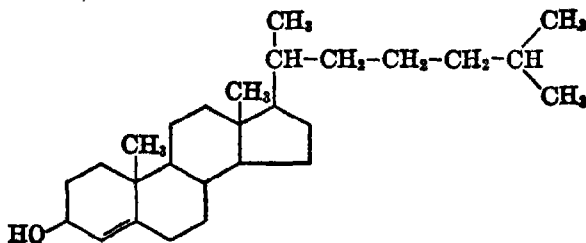
XX. Allocholesterol

Allocholesterol also has been suggested as an intermediary form in the physiological conversion of cholesterol to coprosterol. In agreement with this suggestion, catalytic reduction of allocholesterol gives only coprosterol.[145]

When Δ^4-cholestenone is reduced with sodium, a molecular compound, m.p. 160°, of dihydrocholesterol and *epi*allocholesterol is obtained.[146] The molecular compound was at one time regarded as an isomer of cholesterol and was known as β-cholesterol.

The Cholestadienes. Cholesterol can be dehydrated by distilling from anhydrous copper sulfate or by various other methods. The dehydration product is known as cholesterilene. Bergmann[147] has studied the structure of this compound and from its reactions has concluded that it is a $\Delta^3,^5$-cholestadiene. As proof of the correctness of this formulation $\Delta^3,^5$-cholestadiene was prepared from 7-ketocholesteryl acetate by splitting off acetic acid and reducing the carbonyl at C_7 by the Wolff-Kishner method. In this conversion the carbonyl at C_7 serves to stabilize the double bond at C_5. An isomer of cholesterilene, $\Delta^{2,4}$-cholestadiene, may be obtained by heating cholesterol over aluminum oxide and distilling the product. On treatment with hydrochloric acid, $\Delta^{2,4}$-cholestadiene is rearranged to the $\Delta^3,^5$-diene.*

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[145] Evans, Jr., and Schoenheimer, *J. Am. Chem. Soc.*, **58**, 182 (1936); *J. Biol. Chem.*, **114**, 567 (1936).

[146] Diels and Linn, *Ber.*, **41**, 260 (1908); Evans, Jr., and Schoenheimer, *J. Biol. Chem.*, **115**, 17 (1936); *cf.* reference 95.

[147] Stavely and Bergmann, *J. Org. Chem.*, **1**, 567, 575 (1936). A good bibliography to the earlier work is given in the first of these articles.

* The preparation of a number of other cholestadienes is described by Eck and Hollingsworth, *J. Am. Chem. Soc.*, **64**, 140 (1942).

The two cholestadienes show a number of interesting differences. The physical properties are: $\Delta^{3,5}$-cholestadiene, m.p. 78–79°, $[\alpha]_D -64°$ (CHCl$_3$); $\Delta^{2,4}$-cholestadiene, m.p. 63°, $[\alpha]_D +114°$ (CHCl$_3$). The shift in specific rotation from negative in the $\Delta^{3,5}$-diene to positive in the $\Delta^{2,4}$-diene is further evidence that the assigned structures are correct, since Callow and Young (p. 1378) have noted that compounds with Δ^5-unsaturation show a less positive rotation than those with Δ^4-unsaturation. The $\Delta^{3,5}$-diene adds maleic anhydride (p. 685) with difficulty, and the acid formed by hydrolysis gives insoluble alkaline salts, thus differing from the other known maleic acid addition compounds in this group. On the other hand, the maleic acid addition product of $\Delta^{2,4}$-cholestadiene forms soluble alkaline salts. $\Delta^{3,5}$-Cholestadiene has an absorption spectrum with maxima at 229, 235, and 244 mμ, while that of the $\Delta^{2,4}$-diene is at 260 mμ. On catalytic reduction, $\Delta^{3,5}$-cholestadiene is hydrogenated to cholestane (80 per cent) and coprostane (20 per cent), but $\Delta^{2,4}$-cholestadiene is quantitatively converted into coprostane. When oxygen is passed through an irradiated alcoholic solution of $\Delta^{2,4}$-cholestadiene sensitized with eosin, 2,5-peroxido-Δ^3-cholestene is formed. The peroxide is unstable in sunlight and is rearranged to some uncharacterized peroxide.[148]

Sterols from Lower Forms of Animal Life. From oysters and clams the characteristic sterol, ostreasterol, $C_{29}H_{48}O$, has been isolated.[149] Its structure is not completely known, but hydrogenation converts it to sitostanol [γ-sitostanol(?)]. An ostreasterol II, m.p. 121–122°, has been described also.

The eggs and oil of the silkworm contain a mixture of cholesterol and sitosterol, which at one time was regarded as a definite compound.[150] It is uncertain whether the sitosterol originates from the diet or whether it is synthesized by the worm. These and other sterols from the lower forms of animal and plant life are of interest, as they suggest a relationship between the stage of evolution and the type of sterol formed. Until the role of the sterols is understood, however, it is impossible to evaluate such an evolutionary process if one exists.

In urine there are normally present small amounts of cholesterol [151] and traces of various urinary hydrocarbons.[152] Besides these constitu-

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[148] Butenandt and Kudzus, Z. physiol. Chem., **253**, 1 (1938); Skau and Bergmann, J. Org. Chem., **3**, 166 (1938).

[149] Bergmann, J. Biol. Chem., **104**, 317, 553 (1934).

[150] Bergmann, ibid., **107**, 527 (1934); **117**, 175 (1937).

[151] Butenandt and Dannenbaum, Z. physiol. Chem., **243**, 151 (1937); Marker, J. Am. Chem. Soc., **61**, 1287 (1939).

[152] Hart and Northrup, J. Am. Chem. Soc., **57**, 2726 (1935); Marker, ibid., **60**, 2442 (1938); **61**, 1287 (1939).

ents, α- and β-equistanols, $C_{30}H_{53}OH$, which may be sterols also, are excreted in equine,[153] bovine,[154] and porcine [155] urines. The equistanols may originate from the diet or, since steers [156] do not excrete either form, may be formed in the sex organs. In the lower animal orders, or during pregnancy in higher and lower orders, various polyhydric sterols derived from the hydrocarbons pregnane (p. 1489) and urane (p. 1496) are also excreted. These may be formed from the sex hormone progesterone or from certain of the adrenal substances by reduction *in vivo*; they are discussed later.

The Phytosterols

A large number of plant sterols have been reported in the literature,* but many of them have been so poorly characterized that relatively few can be regarded as chemical individuals. Stigmasterol, $α_1$-sitosterol, β-sitosterol, α-spinasterol, and brassicasterol are the phytosterols which have been studied most carefully.

Stigmasterol. Although stigmasterol is widely distributed in plants both as the free sterol and as glycosides,[157] only soybean and calabar bean oils contain enough of the sterol to be used as sources. From soybean oil, stigmasterol is conveniently separated as its sparingly soluble acetate tetrabromide from the accompanying sitosterols. Its structure (XXI) has been determined as 3(β)-hydroxy-24-ethyl-$Δ^{5,22}$-cholestadiene by Fernholz,[158] building on the earlier work of Guiteras.[159] The methods used were essentially the same as those employed for the determination of the structure of cholesterol, save that the double bond of the side chain was placed through formation of ethylisopropylacetaldehyde by ozonization of the sterol. Among the important derivatives of stigmasterol are the hydrogenated sterols, 22-dihydrostigmasterol and stigmastanol. The former is conveniently obtained by rearranging the parent sterol ethers to *i*-stigmasterol ethers, hydrogenating, and then rearranging and hydrolyzing to 22-dihydrostigmasterol.[160]

The Sitosterols. Originally the principal sterol fraction isolated from plant oils was considered to be "sitosterol." Largely through the

[153] Marker *et al.*, *ibid.*, **60**, 1555 (1938); Marker and Rohrmann, *ibid.*, **61**, 2537 (1939).

[154] Marker, *ibid.*, **60**, 2442 (1938); **61**, 944 (1939).

[155] Marker and Rohrmann, *ibid.*, **61**, 3476 (1939).

[156] Marker, *ibid.*, **61**, 1287 (1939).

* For partial list see "Biochemisches Handlexikon" (1923), **X**, 179; (1933), **XIV**, 826.

[157] *Cf.* Thornton, Kraybill, and Mitchell, *J. Am. Chem. Soc.*, **62**, 2006 (1940), for recent reference.

[158] Fernholz, *Ann.*, **507**, 128 (1933); **508**, 215 (1934); Fernholz and Chakravorty, *Ber.*, **67**, 2021 (1934).

[159] Guiteras, *Z. physiol. Chem.*, **214**, 89 (1933).

[160] Fernholz and Ruigh, *J. Am. Chem. Soc.*, **62**, 3346 (1940).

work of Anderson [161] and of Wallis,[162] this sterol fraction is now known to contain at least five components, α_1-, α_2-, α_3-, β-, and γ-sitosterols. α_1-Sitosterol probably has the structure 3(β)-hydroxy-24-ethyl-$\Delta^{5,8(14)}$-cholestadiene.[162] In line with this formulation, α_1-sitosterol is easily hydrogenated to α_1-dihydrositosterol, which shows the reactions of an α-stenol. On isomerizing with hydrogen chloride and hydrogenating, this α-stenol is converted through a β-stenol to α_1-sitostanol, which
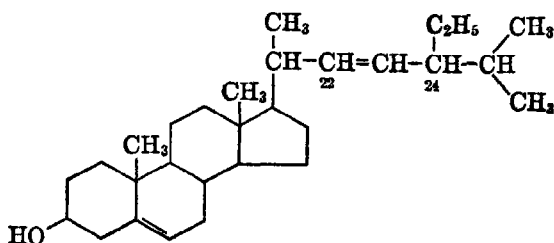
XXI. Stigmasterol

appears to be stereoisomeric in the C_{17} side chain with stigmastanol. Little is known of the structures of α_2- and α_3-sitosterol, but the latter is doubly unsaturated and is precipitated by digitonin.[162] β-Sitosterol has been shown to be 22-dihydrostigmasterol.[162, 163] Recently the sterol from cinchona bark, formerly known as cinchol, has been identified as β-sitosterol.[164] γ-Sitosterol apparently differs from β-sitosterol merely in the spatial configuration of the C_{17} side chain.[162]

The Spinasterols. From spinach, and also from alfalfa, the characteristic sterol, α-spinasterol (XXII), has been isolated.[165] In spinach this sterol is accompanied by the allied compounds, β- and γ-spinasterol. The structure of α-spinasterol has been shown by Fernholz to be 3(β)-hydroxy-24-ethyl-$\Delta^{8(14),22}$-cholestadiene. This sterol and zymosterol (p. 1399) are the only known natural unsaturated sterols that do not have an ethenoid bond at C_5.

[161] Anderson and Shriner, *ibid.*, **48**, 2976 (1926) ; Anderson, Shriner, and Burr, *ibid.*, **48**, 2987 (1926). *Cf.* Ruzicka and Eichenberger, *Helv. Chim. Acta*, **18**, 430 (1935).

[162] Fernholz and Wallis, *J. Am. Chem. Soc.*, **58**, 2446 (1936) ; Wallis and Chakravorty *J. Org. Chem.*, **3**, 335 (1937) ; Bernstein and Wallis, *ibid.*, **3**, 341 (1937) ; *J. Am. Chem. Soc.*, **61**, 1903, 2309 (1939).

[163] Bengtsson, *Z. physiol. Chem.*, **237**, 46 (1935).

[164] Hesse, *Ann.*, **228**, 294 (1885) ; Liebermann, *Ber.*, **17**, 871 (1884) ; **18**, 1805 (1885) ; Windaus and Deppe, *Ber.*, **66**, 1689 (1933) ; Dirscherl, *Z. physiol. Chem.*, **235**, 1 (1935) ; **257**, 239 (1938).

[165] Hart and Heyl, *J. Biol. Chem.*, **95**, 311 (1932) ; Heyl and Larsen, *J. Am. Chem. Soc.*, **56**, 942 (1934) ; Larsen and Heyl, *ibid.*, **56**, 2993 (1934) ; Larsen, *ibid.*, **60**, 2431 (1938) ; Simpson, *J. Chem. Soc.*, 730 (1937) ; Fernholz and Moore, *J. Am. Chem. Soc.*, **61**, 2467 (1939) ; Fernholz and Ruigh, *ibid.*, **62**, 2341 (1940) ; King and Ball, Jr., *ibid.*, **61**, 2910 (1939)

XXIII. α-Spinasterol
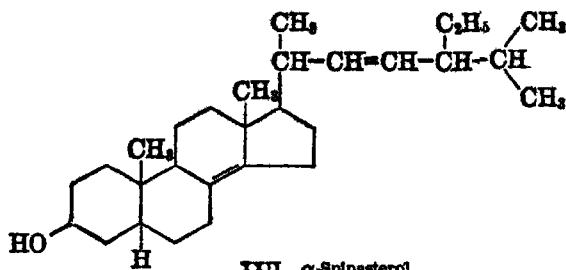
Miscellaneous Plant Sterols. In addition to the above plant sterols there are a number of others which have been investigated. Noteworthy among these are brassicasterol from rapeseed oil and fucosterol from bladder wrack. Brassicasterol has the structure 3(β)-hydroxy-24-methyl-$\Delta^{5,22}$-cholestadiene and is exceptional in that it has a methyl group at C_{24} rather than the ethyl group usually found at this position in plant sterols.[166] Fucosterol contains two double bonds and, on catalytic hydrogenation, is reduced to stigmastanol.[167] One of the double bonds of fucosterol is probably at C_5, but the position of the other is uncertain.

Further work with plant sterols may show new variations in the sterol architecture. Among those currently receiving attention are bessisterol, $C_{29}H_{50}O$, from *coicis* seeds,[168] and cafesterol, $C_{20}H_{28}O_3$, from coffee oil.[169] The former may be related to allocholesterol,* and the latter may be devoid of the C_{17} side chain.

The Mycosterols

The sterols of yeast have been studied with moderate thoroughness, while those of the fungi have been poorly investigated. In general, sterols are not present in bacteria, but small amounts of an unknown sterol have been demonstrated in *Azobacter chroococcum*.[170]

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[166] Windaus and Welsch, *Ber.*, **42**, 612 (1909); Fernholz and Stavely, *J. Am. Chem. Soc.*, **61**, 142 (1939); **62**, 428 (1940).

[167] Heilbron and co-workers, *J. Chem. Soc.*, 1572 (1934); 1205 (1935); 738 (1936). *Cf.* Larsen, *J. Am. Chem. Soc.*, **60**, 2431 (1938).

[168] Kuwada and Yosiki, *J. Pharm. Soc. Japan*, **59**, 203, 282 (1939) [*Chem. Zentr.*, (I) 2316 (1940)]; *ibid.*, **60**, 25 (1940) [*Chem. Zentr.*, (II) 630 (1940)].

[169] Slotta and Neisser, *Ber.*, **71**, 1991, 2342 (1938); Hauptmann and Franca, *Z. physiol. Chem.*, **259**, 245 (1939); Miescher *et al.*, *Helv. Chim. Acta*, **24**, 332E (1941).

* Various derivatives of bessisterol have been found to be identical with those from the spinasterols by Kuwada and Yosiki, *J. Pharm. Soc. Japan*, **60**, 407 (1940)[*C. A.*, **35**, 461 (1941)].

[170] Anderson, Schoenheimer, Crowder, and Stodola, *Z. physiol. Chem.*, **237**, 40 (1935); Sifferd and Anderson, *ibid.*, **239**, 270 (1936).

Ergosterol, the principal mycosterol, is found both in yeast and in fungi. In the former it is accompanied by small amounts of α-dihydroergosterol, zymosterol, and cerevisterol, a polyhydroxy sterol.[171] Fungisterol, $C_{28}H_{44}O$, has been isolated from several kinds of fungi.[172] Present in the sterol fraction from yeast is a so-called cryptosterol, $C_{30}H_{50}O$, which forms an insoluble digitonide but which is not a sterol.[173] There is some question whether cerevisterol and fungisterol are sterols.

Zymosterol. Through the work of Heilbron[171] the structure of the difficultly accessible zymosterol has been established as 3(β)-hydroxy-$Δ^{8(14),24}$-cholestadiene. The absence of a 5 : 6-ethenoid linkage correlates this sterol with α-spinasterol, but the occurrence of unsaturation at C_{24} : C_{25} is unique and without parallel in steroid chemistry.

Ergosterol. Apparently pure ergosterol was obtained by Tanret[172] first in 1908, although he doubtless had nearly pure preparations some time before this.[174] The sterol attracted little attention until 1926–1927, when it was discovered that irradiation with ultra-violet light converts it into a vitamin D. Subsequent work has shown that ergosterol is the principal yeast sterol. The content in yeast varies considerably in the different species[175] and is influenced greatly by the nature of the substrate on which the yeast is cultured.[176] The structure of ergosterol (XXIII), as 3(β)-hydroxy-24-methyl-$Δ^{5,7,22}$-cholestatriene, has been determined principally by Windaus and his school at Göttingen. Because of the importance of this sterol, the details of the investigation follow:

1. The structure of the side chain was established by studying the action of ozone on ergosterol,[100] and the products of chromic acid oxidation of a partially reduced ergosterol.[177] In this way a double bond at C_{22} and a methyl group at C_{24} were placed through the isolation of isopropylacetaldehyde and of thujaketone

$$CH_3COCH_2CH_2CH(CH_3)CH(CH_3)_2$$

respectively.

[171] Smedley-Maclean, *Biochem. J.*, **14**, 484 (1920); **22**, 22, 980 (1928); Callow, *ibid.*, **25**, 87 (1931); Honeywell and Bills, *J. Biol. Chem.*, **99**, 71 (1932); **103**, 515 (1933); Wieland and Kanaoka, *Ann.*, **530**, 146 (1937); Heath-Brown, Heilbron, and Jones, *J. Chem. Soc.*, 1482 (1940).

[172] Tanret, *Compt. rend.*, **147**, 75 (1908); *Ann. chim. phys.*, **8**, 15, 313 (1908); *cf.* Ratcliffe, *Biochem. J.*, **31**, 240 (1937).

[173] Wieland, Pasedach, and Ballauf, *Ann.*, **529**, 68 (1937); Wieland and Joost, *Ann.*, **546**, 103 (1941).

[174] Tanret, *Compt. rend.*, **108**, 98 (1889); *Ann. chim. phys.*, **6**, 20, 289 (1890).

[175] Heiduschka and Lindner, *Z. physiol. Chem.*, **181**, 16 (1929).

[176] Bills and co-workers, *J. Biol. Chem.*, **87**, 259 (1930); **94**, 213 (1931).

[177] Windaus, Werder, and Gschaider, *Ber.*, **65**, 1006 (1932).

2. For the determination of the nature of the nucleus, and of the position of the hydroxyl, it was necessary to have the completely saturated ergostanol. This was finally achieved by Reindel [178] through catalytic reduction of ergosterol with the Adams catalyst. From the saturated sterol the hydrocarbon ergostane was prepared. Chromic acid oxidation of ergostanyl acetate (XXIV) gave 3(β)-hydroxynor*allo*cholanic acid (XXV); [179] of ergostane gave nor*allo*cholanic acid. [180] These two transformations determined the nature of the nucleus, placed the hydroxyl at C_3, and confirmed hypotheses based on the study of the dicarboxylic acids obtained by opening ring A. [181]

XXIII. Ergosterol

XXIV. Ergostanyl acetate

XXV. 3(β)-Hydroxynor*allo*cholanic acid
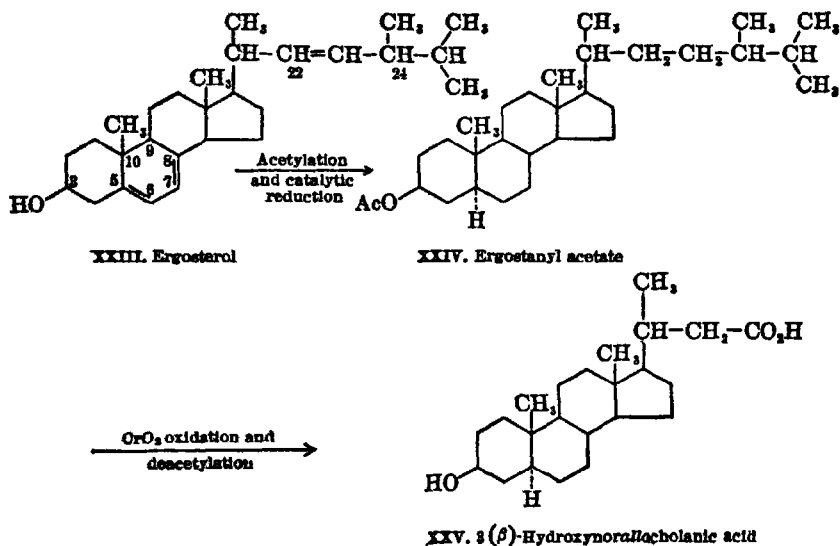
3. The C_3—OH group was shown to be part of an α,γ-system with an ethylenic bond at C_5 by a series of reactions [182] exactly paralleling those for cholesterol (transformation of cholesterol to cholestanetriol, etc., p. 1354).

4. The absorption spectrum of ergosterol [113] and the molecular refraction [183] indicated that a pair of double bonds was present as a conjugated system. This was supported by the fact that ergosteryl acetate forms addition products (adducts) with maleic and citraconic anhydrides. [184]

[178] Reindel and Walter, *Ann.*, **460**, 212 (1928).
[179] Fernholz and Chakravorty, *Ber.*, **67**, 2021 (1934).
[180] Chuang, *Ann.*, **500**, 270 (1933).
[181] Reindel, *Ann.*, **466**, 131 (1928).
[182] Windaus, Inhoffen, and v. Reichel, *Ann.*, **510**, 248 (1934).
[183] v. Auwers and Wolter, *Nachr. Ges. Wiss. Göttingen*, 101 (1931).
[184] Alder, in "Handbuch der biologischen Arbeitsmethoden," Urban and Schwarzenberg, Berlin (1933), Abt. 1, Teil 2, Hälfte 2, Band 2, p. 3138.

That the adducts were formed through reaction with the conjugated system in the nucleus was established by ozone treatment and by reduction to dihydro compounds. From the latter, the maleic and citraconic anhydrides were removed by sublimation. The properties of the product, 22-dihydroergosteryl acetate, also indicated the presence of a conjugated system.[185]

5. A clue to the position of the conjugated system in ergosterol was obtained by studying the action of fuming nitric acid. From this reaction mixture, toluenetetracarboxylic acid was obtained.[100] The reaction is explicable by assuming the conversion of a partially unsaturated to a benzenoid ring, and the wandering of a methyl group. Although the mechanism is not wholly clear, the production of this acid placed the conjugated system in ring B or C, or possibly distributed between the two, and excluded the possibility of its being contained in ring A or D.

6. Further evidence for the position of the conjugated system was obtained by studying neoergosterol. Like the other dehydrosterols, ergosterol forms a pinacol[186] when irradiated with visible light in the presence of a sensitizer and in the absence of oxygen. Pyrolysis of the pinacol splits off methane and produces neoergosterol, $C_{27}H_{40}O$ (XXVI).[187] This sterol contains one reactive double bond, which is

XXVI. Neoergosterol

XXVII. Benzenetetracarboxylic acid

XXVIII. Dehydroneoergosterol
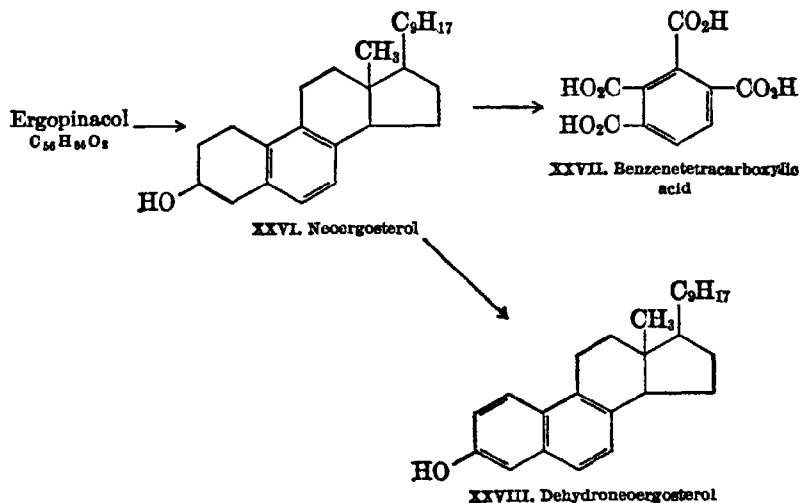
present in the side chain, since ozonization of the sterol produces methylisopropylacetaldehyde.[188] This suggests that by the loss of methane the

[185] Windaus and Langer, *Ann.*, **508**, 105 (1933).
[186] Windaus and Borgeaud, *Ann.*, **460**, 235 (1928).
[187] Bonstedt, *Z. physiol. Chem.*, **185**, 165 (1929).
[188] Inhoffen, *Ann.*, **497**, 130 (1932).

ring containing the conjugated system is converted to a benzenoid structure. Proof of this was obtained by the action of fuming nitric acid on,[188] and by catalytic dehydrogenation of,[189] neoergosterol. From the fuming nitric acid reaction mixture, benzenetetracarboxylic acid (XXVII) was isolated; in neoergosterol a methyl group is therefore no longer attached to the ring, which normally is converted to toluene-tetracarboxylic acid. The catalytic dehydrogenation with platinum black of neoergosterol gave a phenol, dehydroneoergosterol (XXVIII). Zelinsky[190] has shown that dehydrogenation of a cyclohexane ring by the action of platinum black takes place only when no quaternary carbon atom is present. Thus a methyl group is not present at C_{10} in neoergosterol. These reactions limited still further the position of the conjugated system to rings B and C.

7. The position of the conjugated system was finally placed at 5 : 6 · 7 : 8 by studying ergostadienetriol.[191] By the action of perbenzoic acid, ergosterol was converted into an oxide, which on hydrolysis gave ergostadienetriol (XXIX), containing two secondary and one tertiary hydroxyl groups.* On reduction, the dienetriol was converted to ergostanetriol (XXX), which gave the reactions of an α-glycol when tested by the lead tetraacetate method of Criegee.[192]   Evidently, 1,2-addition of

XXIX. Ergostadienetriol   XXX. Ergostanetriol
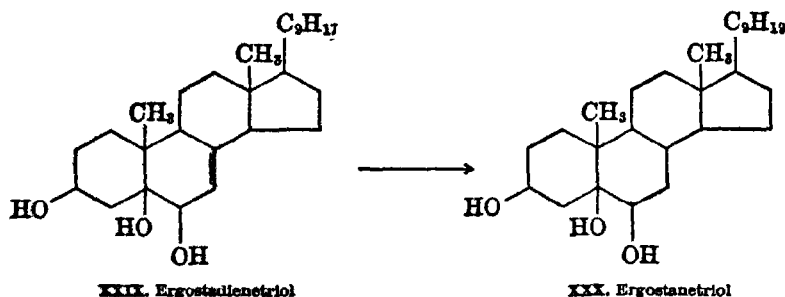
oxygen to the double bond at C_5 is the preliminary stage in the formation of the α-glycol. The other possible position for the conjugated system, 6 : 7 · 8 : 9, is definitely excluded, for only by 1,4-addition could an additional secondary and a new tertiary hydroxyl group be introduced if such a system were present, and the resulting compound would not behave like an α-glycol.

[188] Honigmann, Ann., 511, 292 (1934).
[189] Zelinsky, Ber., 44, 3121 (1911); 45, 3678 (1912); 56, 1716 (1923).
[191] Windaus and Lüttringhaus, Ann., 481, 119 (1930); Windaus, Inhoffen, and v. Reichel, Ann., 510, 248 (1934). Cf. Heilbron and co-workers, J. Chem. Soc., 1410 (1933).
    * Actually two isomers are produced as with the cholestanetriols, p. 1355.
[192] Criegee, Kraft, and Rank, Ann., 507, 159 (1933).

Isomerization of Ergosterol. By a variety of procedures ergosterol can be isomerized. Because of partial reduction, epimerization of the C_3—OH by treatment with sodium ethoxide cannot be employed, but can be used on certain derivatives of ergosterol. The other methods which have been used—the action of hydrogen chloride, subtraction and addition of water or hydrogen, addition and subtraction of hydrogen, and irradiation with ultra-violet light in the absence of oxygen—evidently produce a shift of the double bonds or of the hydroxyl group or both.[192] Alder[194] and Fernholz[194] have tabulated the several isomers, but only the changes produced by irradiation will be discussed here.

Irradiation Products of Ergosterol. The transformation that takes place when ergosterol is irradiated may be schematically represented:[195]

$$\text{Ergosterol} \rightarrow \text{Lumisterol} \rightarrow \text{Tachysterol} \rightarrow \text{Vitamin D}_2 \underset{\text{(Calciferol)}}{\overset{\nearrow \text{Suprasterol I}}{\underset{\searrow \text{Toxisterol}}{\longrightarrow \text{Suprasterol II}}}}$$
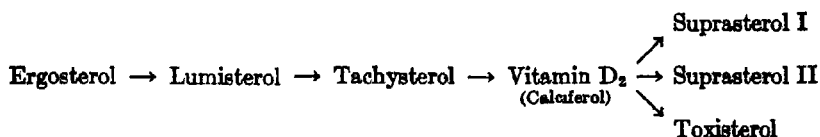
All the irradiation products have the same side chain as ergosterol, for ozonization produces methylisopropylacetaldehyde in each case.[100] Since none of the reaction products forms an insoluble digitonide, it was at one time thought that the first change was epimerization of the C_3—OH. It is now evident that this is incorrect, and that probably the initial effect of irradiation is a spatial rearrangement of the C_{10}—CH_3. The photochange appears to be quite general for 7-dehydrosterols, for analogous irradiation products are known for several of the homologs of ergosterol.

Lumisterol. Of the irradiation products, only lumisterol (XXXI) gives Diels' hydrocarbon when dehydrogenated with selenium,[196] and
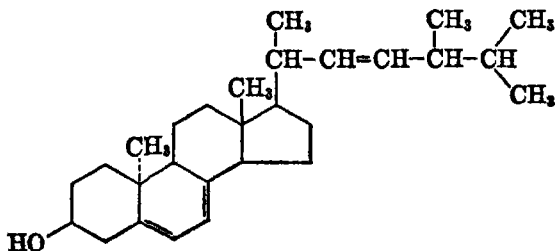
XXXI. Lumisterol

[193] Lettré and Inhoffen give a flow sheet of these isomers in "Über Sterine, Gallensäuren und verwandte Naturstoffe," Enke, Stuttgart (1936), p. 131.

[194] Fernholz, Tabulae Biologicae Periodicae, 3, 196 (1933).

[195] Lettré, Ann., 511, 280 (1934); Dimroth, Ber., 70, 1631 (1937).

[196] Dimroth, Ber., 68, 539 (1935); Müller, Z. physiol. Chem., 233, 223 (1935).

toluenetetracarboxylic acid when oxidized with fuming nitric acid.[197] Although lumisterol does not form a pinacol, there appears to be the same conjugated system of double bonds in ring B as in ergosterol.[198] Both ergosterol [199] and lumisterol [199] are dehydrogenated by mercuric acetate to give dehydro compounds in which the new double bond is probably at $C_9 : C_{11}$. The two compounds have the same absorption spectra, but on catalytic hydrogenation dehydrolumisterol yields perhydropyrocalciferol.[200] As is shown later, pyrocalciferol is a compound in which ring B has been opened and then closed by pyrolysis. Clearly lumisterol originates by a photochange in the neighborhood of the linkage C_9—C_{10}, and the only change that can be reconciled with the other facts is a rearrangement involving the C_{10}—CH_3 group. In accord with this the *epi* compounds of lumisterol and of the hydrolumisterols give insoluble digitonides.

Tachysterol. Lumisterol and tachysterol form maleic and citraconic anhydride adducts readily. Indeed, the ease of adduct formation with tachysterol is so great that its name (Gr. *tachys*, swift) is derived from this fact. Comparative hydrogenation of the citraconic anhydride adducts, or perbenzoic acid titration of the dinitrotoluyl esters of tachysterol and dehydroergosterol, shows an equal degree of unsaturation in the two compounds.[195] Since dehydroergosterol contains four double bonds, tachysterol must be equally unsaturated. But tachysterol is isomeric with ergosterol, and a fourth double bond can be accommodated only by the opening of one of the rings, presumably ring B.
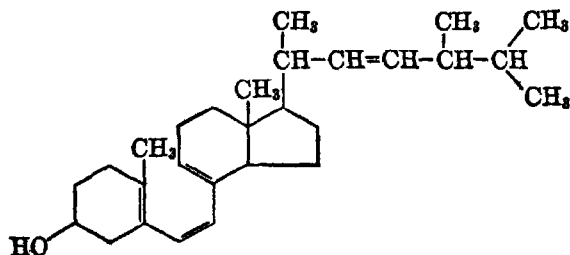
XXXII. Tachysterol
(Provisional)

Since the adducts of tachysterol cannot be smoothly ozonized or oxidized with potassium permanganate, it seems probable that the arrangement of the double bond is that shown in structure XXXII.[201]

[197] Guiteras, Nakamiya, and Inhoffen, *Ann.*, **494**, 122 (1932).

[198] Heilbron, Spring, and Stewart, *J. Chem. Soc.*, 1221 (1935); Heilbron, Moffet, and Spring, *ibid.*, 411 (1937); Kennedy and Spring, *ibid.*, 250 (1939).

[199] Müller, reference 196.

[200] Dimroth, *Ber.*, **69**, 1123 (1936).

[201] Grundmann, *Z. physiol. Chem.*, **252**, 151 (1938).

Vitamin D. The story of vitamin D—the discovery and the chemical characterization of the antirachitic substances produced by irradiating foodstuffs or sterols with ultra-violet light—is one of the scientific classics.[202] In 1924 it was noted (Steenbock; Hess) that irradiation of foodstuffs with ultra-violet light produced antirachitic properties. Subsequently the fats were studied, and a clue to the nature of the provitamin was obtained when it was shown (Steenbock; Hess; Rosenheim) that irradiation of the sterols resulted in very potent preparations. At first cholesterol appeared to be the precursor of the potent substance, but it was soon found (Hess; Rosenheim; Heilbron) that the activity of the irradiated product depended upon the content in cholesterol of a small amount of impurity with the absorption spectrum characteristic of ergosterol. This was taken (Rosenheim and Webster; Windaus and Hess) to indicate that ergosterol was the provitamin D in cholesterol, and the view seemed to be justified when finally in 1931 a very potent crystalline compound, vitamin D_2, was isolated from the irradiation products of ergosterol. For a time vitamin D_2 was thought to be identical with the natural vitamin D of fish-liver oils, but bioassays showed that their physiological properties were not the same. The discrepancy led in part to the study of the irradiation products obtained from other dehydrosterols, and it is now established that the compound obtained by irradiating 7-dehydrocholesterol is identical with one of the natural vitamins D. From the résumé above it is apparent that there are several substances with vitamin D activity which may be produced by artificial means, and from the bioassays discussed below there is evidence that fish-liver oils contain more than one natural vitamin D.

The compound now known as vitamin D_2 * or calciferol was isolated almost simultaneously by Angus, Askew, Bourdillon, et al.,[203] and by Windaus and co-workers,[204] both groups at first obtaining a substance (calciferol, old; vitamin D_1) which was later found to be an addition product of lumisterol and vitamin D_2. By irradiating with a magnesium arc, vitamin D_2, practically free of lumisterol, was obtained by Windaus, and, at the same time, the English group found that calciferol could be separated from lumisterol by crystallization of the dinitrobenzoyl esters. Vitamin D_2 has the following properties: m.p. 115–117°, $[\alpha]_D$ + 103°

[202] Reed, Struck, and Steck, "Vitamin D," University of Chicago Press, Chicago (1939); Rosenberg. "Chemistry and Physiology of the Vitamins," Interscience Publishers, New York (1942).

* Windaus introduced the practice of designating the different vitamins by means of subscripts.

[203] Askew and co-workers, *Proc. Roy. Soc. (London),* **107B,** 76 (1930); Angus and co-workers, *ibid.,* **108B,** 340 (1931); Askew and co-workers, *ibid.,* **109B,** 488 (1932).

[204] Windaus and co-workers, *Ann.,* **489,** 252 (1931); **492,** 226 (1932). *Cf.* Lüttringhaus in "Fortschritte der physiol. Chem., 1929–1934," Verlag Chemie, Berlin (1934), p. 266.

(alc.), potency per mg. = 40,000 international antirachitic units (I.U.). Although the pure vitamin is stable for months at 37°, oil solutions in contact with air are somewhat unstable, and the "half life" of an olive oil solution under these conditions is about 3 years.[205] Physiologically, vitamin D_2 and the other compounds with vitamin D activity regulate the phosphorus-calcium metabolism, but how they function is unknown.[206]

The other products formed by irradiation of ergosterol are not antirachitic, but tachysterol and toxisterol can raise a low blood-calcium level to normal. The derivative dihydrotachysterol has been introduced into clinical medicine for this purpose under the designation A.T. 10 (A.T. = antitetanus).[207]

During the period that vitamin D_2 was being studied chemically, evidence was accumulating from biological work to show that the natural vitamin D of fish oils was different from vitamin D_2, and that vitamin D activity could be conferred on cholesterol or on some impurity in cholesterol.[208] The differences were definitely established when Waddell[209] showed in 1934 that preparations of vitamin D_2 and of natural vitamin D, of equal potency in rats, did not have the same potency when tested on chicks, the natural being far more potent. Shortly before this it was discovered that 22-dihydroergosterol could be converted by irradiation to a substance with antirachitic properties,[210] and subsequently this irradiation product was isolated in pure form.[211] It is now known as vitamin D_4 and has the properties: m.p. 107–108°; $[\alpha]_D + 89.3°$ (acetone); potency/mg. = 20,000–30,000 I.U. These two stimuli aroused interest again in cholesterol as a precursor of the natural vitamin D. It was soon found that cholesterol may be converted via 7-dehydrocholesterol (XXXIII) to an antirachitic substance, vitamin D_3, with the same rat:chick assay as natural vitamin D concentrates.[212]

The inference that vitamin D_3 is the principal natural vitamin has been confirmed by its isolation from fish-liver oil and by the isolation of 7-dehydrocholesterol, provitamin D_3, from the sterols of the skin.[213] Prior to 1936, attempts to isolate the active constituents of fish-liver oil had led

[205] Bourdillon, Bruce, and Webster, Biochem. J., 26, 522 (1932).

[206] Bills, Physiol. Rev., 15, 1 (1935).

[207] Werder, Z. physiol. Chem., 260, 119 (1939).

[208] Bills, Cold Spring Harbor Symposia Quant. Biol., 3, 328 (1935).

[209] Waddell, J. Biol. Chem., 105, 711 (1934).

[210] Windaus and Langer, Ann., 508, 105 (1933).

[211] Windaus and Trautmann, Z. physiol. Chem., 247, 185 (1937).

[212] Windaus, Lettré, and Schenck, Ann., 520, 98 (1935); Windaus, Schenck, and v. Werder, Z. physiol. Chem., 241, 100 (1936); Grab, ibid., 243, 63 (1936).

[213] Windaus and Bock, Z. physiol. Chem., 245, 168 (1937) cf. Boer, Reerink, van Wijk and van Niekerk, Proc. Koninkl. Akad. Wetenschappen Amsterdam, 39, 622 (1936).

to concentrates of high potency, but never to the vitamin itself. In 1936, Brockmann isolated vitamin D_3 from concentrates of tuna-liver oil,[214] and in 1937 from halibut-liver oil.[215] The isolation was effected through a combination of chromatographic analysis of the crude vitamin preparation, followed by crystallization and chromatographic analysis of the 3,5-dinitrobenzoate of the vitamin. In its chemical and physiological properties the natural vitamin D_3 agrees with the product obtained by irradiating 7-dehydrocholesterol. The accepted constants for vitamin D_3 are m.p. $82-85°$; $[\alpha]_D + 83.3°$ (acetone); potency/mg. $= 40,000$ I.U.[216]
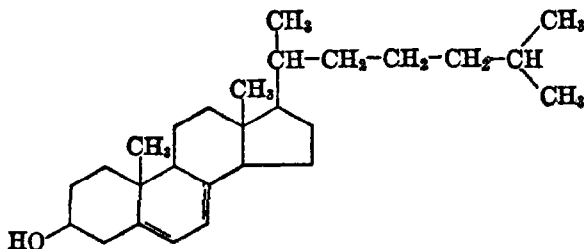
XXXIII. 7-Dehydrocholesterol

The vitamin D content of fish-liver oil varies widely with species, and usually contains small amounts of vitamin D_2.[217] The liver oils from the members of the percomorph family (mackerel, etc.) are far more potent than those from halibut or cod. The varying content of vitamin D in the fish oils may be related more to dietary habits, however, than to species.

The occurrence of vitamin D in fish-liver oil is puzzling. It seems improbable that fish can synthesize either vitamin D_2 or D_3, and it is possible that the vitamins originate from the primary foodstuffs of fish, zoöplankton and algae, which are known to contain small amounts of antirachitic vitamins. Even more puzzling is the presence of provitamin D_2 or D_3 in the walls of the alimentary canal of certain invertebrates. In earthworms and red snails a large percentage of the total sterol is ergosterol, while in the horned snail 7-dehydrocholesterol predominates.[218]

Vitamin D_2. The structure of vitamin D_2 (XXXIV) has been determined by study of the products of oxidative degradation of the vitamin or of the dihydrocalciferol maleic anhydride adduct. Previous to

[214] Brockmann, Z. physiol. Chem., **241**, 104 (1936); Brockmann and Chen, ibid., **241**, 129 (1936). Cf. Simons and Zueker, J. Am. Chem. Soc., **58**, 2655 (1936).
[215] Brockmann, ibid., **245**, 96 (1937); Brockmann and Busse, ibid., **249**, 176 (1937).
[216] Schenck, Naturwissenschaften, **25**, 159 (1937).
[217] Brockmann and Busse, Z. physiol. Chem., **256**, 252 (1938). Cf. Bills, Massengale, and Imboden, Science, **80**, 596 (1934); Bills and co-workers, J. Nutrition, **13**, 435 (1937).
[218] Bock and Wetter, Z. physiol. Chem., **256**, 33 (1938).

this work it was known from microcatalytic hydrogenation [219] that vitamin D_2 contains four double bonds of which three can be detected by titration with perbenzoic acid,[220] and that reduction with sodium and ethyl alcohol gives a dihydrovitamin that perbenzoic acid titration shows to have three double bonds.[220] With maleic anhydride, calciferyl acetate forms two isomeric α- and β-adducts which are easily reduced to dihydro compounds (XXXV) in which the side chain is saturated. These vitamin and dihydrovitamin adducts are much more stable than those from ergosterol and tachysterol, and they may be distilled without decomposition. Ozone, acting on the dihydro adducts, degrades them to a ketone (XXXVI), $C_{19}H_{34}O$.[221] The structure of this ketone is arrived at indirectly. Selenium dehydrogenation of the dihydro adduct forms 2,3-dimethylnaphthalene, and palladium dehydrogenation yields β-naphthoic acid and naphthalene. These dehydrogenation products must originate from ring A and the ring system produced in adduct formation. The production of 2,3-dimethylnaphthalene and of β-naphthoic acid is particularly significant, since their formation shows the course of the adduct formation to be by addition at C_6 and C_{18}.[221] Since the selenium dehydrogenation to 2,3-dimethylnaphthalene results in the reduction of carboxyl to methyl groups, and this kind of dehydrogenation was unique at the time, the result has been checked by the study of a number of model dehydrogenations.[222]

Further evidence for structure XXXIV has been obtained by direct oxidation of vitamin D_2 with cold chromic acid or permanganate to an oily aldehyde (XXXVII), $C_{21}H_{34}O$.[223] The semicarbazone of this aldehyde absorbs in the ultra-violet with a maximum at 275 mμ, as do other semicarbazones of α,β-unsaturated aldehydes. Under other conditions permanganate oxidation yields the Δ^{22}-unsaturated ketone corresponding to XXXVI.* Ozone oxidation of vitamin D_2 gives as high as 30 per cent of the theoretical formaldehyde formed by the rupture of the methylene linkage at $C_{10} : C_{18}$. This last bit of evidence is not free from objection, however, since similar treatment of ergosterol gives small amounts of formaldehyde.[224]

Transformation Products of Vitamin D_2. When vitamin D_2 is heated for four hours at 180°, its potency is completely destroyed and two pyro-

[219] Kuhn and Möller, *Angew. Chem.*, **47**, 145 (1934).

[220] Windaus, Linsert, Lüttringhaus, and Weidlich, *Ann.*, **492**, 226 (1932).

[221] Windaus and Thiele, *Ann.*, **521**, 160 (1935).

[222] Thiele and Trautmann, *Ber.*, **68**, 2245 (1935).

[223] Heilbron, Jones, Samant, and Spring, *J. Chem. Soc.*, 905 (1935).

* On treatment of this ketone with acid or alkali irreversible rearrangement occurs, indicating conversion of a *trans* to a *cis* form. According to Dimroth and Jonsson, *Ber.*, **74**, 520 (1941), this change confirms the *trans* relationship of rings C/D (p. 1371).

[224] Windaus and Grundmann, *Ann.*, **524**, 295 (1936).

XXXVI

XXXV. Dihydrocalciferol maleic anhydride adduct

β-Naphthoic acid
and naphthalene

2,3-Dimethyl-
naphthalene

Dehydrogenation

Pd

Se

Acetylation, adduct formation
with maleic anhydride, and
catalytic reduction

Cold CrO₃
or KMnO₄

XXXVII

XXXIV. Calciferol
(vitamin D₂)

Formaldehyde
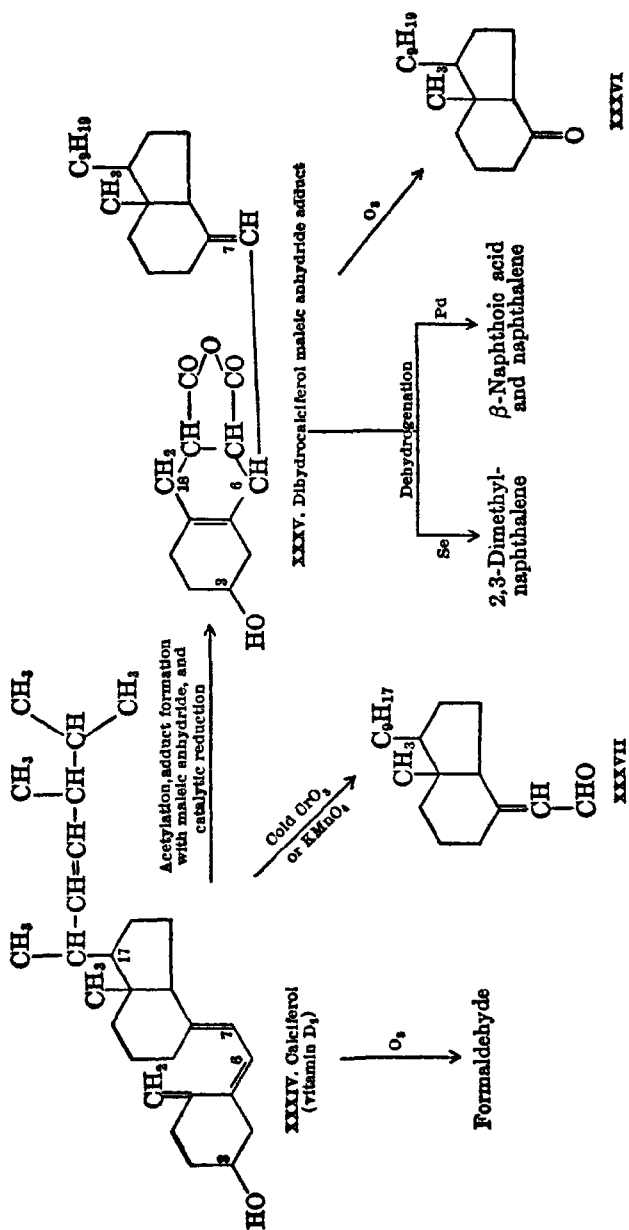
isomers, isopyrovitamin D_2[204] and pyrocalciferol,[203] are produced. The nature of this isomerism has been examined by Windaus.[225] Both these compounds are triply unsaturated and give Diels' hydrocarbon when dehydrogenated with selenium, or toluenetetracarboxylic acid when oxidized with nitric acid.[196] Isopyrovitamin D_2 forms an insoluble digitonide, but pyrocalciferol does not. If isopyrovitamin D_2 is dehydrogenated by treatment with mercuric acetate or perbenzoic acid, dehydroergosterol is obtained. Pyrocalciferol, on the other hand, is dehydrogenated by mercuric acetate to dehydrolumisterol. Since dehydrogenation with mercuric acetate destroys the asymmetry of C_9 through the introduction of a double bond at $C_9 : C_{11}$, it follows that ergosterol and isopyrocalciferol are stereochemically similar about C_{10}, but dissimilar about C_9. Lumisterol and pyrocalciferol are likewise similar at C_{10} and dissimilar at C_9. As the previous discussion has shown, lumisterol differs from ergosterol in the steric position of the C_{10}—CH_3 group, and it is improbable that epimerization of the C_3—OH group has occurred in any of these compounds. Thus the interrelationship about the centers of asymmetry, C_9 and C_{10}, has been represented by Windaus as follows:

	C_9	C_{10}		C_{10}
Ergosterol	+	+	Dehydroergosterol	+
Isopyrovitamin D_2	−	+		
Lumisterol	+(?)	−	Dehydrolumisterol	−
Pyrocalciferol	−(?)	−		

Kennedy and Spring[198] have examined pinacol formation and arrived at different conclusions. Ergosterol and pyrocalciferol, in the form of their acetates, readily form pinacols, while isopyrocalciferol and lumisterol do not. The English workers argue that this indicates a *trans* relationship about the centers C_9 and C_{10} in the first pair, and a *cis* relationship about these same centers in the second pair.

Inversion of the C_9—H in these pyroisomers does not interfere with their ability to undergo photoisomerization when irradiated with ultraviolet light. The photoproducts are not antirachitic, however, and the photoisomerization may be reversed merely by heating in the absence of air. The photoproducts do not absorb in the ultra-violet, and it is possible that the conjugated system has been destroyed by a shift of the 5 : 6 double bond to the 4 : 5 position.[226]

Irradiation of vitamin D_2 destroys its potency and converts it into a mixture of suprasterols I and II, and a poorly defined substance,

[225] Windaus and Dimroth, *Ber.*, **70**, 376 (1937).
[226] Dimroth, *Ber.*, **70**, 1631 (1937).

toxisterol.[227] Suprasterols I and II differ in that the former contains three, and the latter four, double bonds.[196]

Structure and Antirachitic Activity. Structurally the irradiation products from all the 7-dehydrosterols are similar to the corresponding products from ergosterol.[228] In many instances the individual compounds have not been isolated, but enough work has been done on the vitamin D group that some generalizations on structure and antirachitic activity may be made. The irradiation products from ergosterol, 7-dehydrocholesterol, 22-dihydroergosterol, and 22,23-oxidoergosterol [229] have activities of the same order of magnitude, diminishing in the order given; that from 7-dehydrositosterol [230] has only slight activity; and those from 7-dehydrostigmasterol [231] and from $\Delta^{5,7}$-androstadiene-3,17-diol [232] are inactive. Thus, the structure of the C_{17} side chain is one of the principal factors in antirachitic activity. Epimerization of the C_3—OH of vitamin D_2 or D_3,[288] or conversion of this hydroxyl group of vitamin D_2 to carbonyl,[234] greatly diminishes the activity but does not destroy it completely. As cited above, inversion of the C_9—H in ergosterol, and presumably in the other 7-dehydrosterols, prevents the normal course of irradiation and inhibits production of antirachitic activity completely.

There are probably compounds with antirachitic properties other than those described above, all of which result from ultra-violet irradiation of 7-dehydrosterols. Some of the possibilities that must be considered are the antirachitic substances that have been produced in cholesterol by the action of fuller's earth,[235] butyl nitrite,[208] etc. These substances may fit into or modify the general structural pattern that is implied from the structure of vitamin D_2.

THE BILE ACIDS

Most of the bile acids isolated from nature are hydroxy derivatives of cholanic acid (I). The position and number of these hydroxyl groups

[227] Laquer and Linsert, *Klin. Wochschr.*, **12**, 753 (1933).

[228] 7-Dehydrocholesterol: Windaus and co-workers, *Ann.*, **533**, 118 (1937); **537**, 1 (1938); *Z. physiol. Chem.*, **260**, 181 (1939). 22-Dihydroergosterol: Windaus and Güntzel, *Ann.*, **538**, 170 (1939).

[229] Windaus, Linsal, and Buchholz, cited by Dimroth and Paland, reference 232.

[230] Wunderlich, *Z. physiol. Chem.*, **241**, 116 (1936).

[231] Linsert, *ibid.*, **241**, 125 (1936).

[232] Dimroth and Paland, *Ber.*, **72**, 187 (1939). This compound differs from ergosterol in that the C_{17} side chain is replaced by hydroxyl.

[233] Windaus and Naggats, *Ann.*, **542**, 204 (1939); Windaus and Buchholz, *Ber.*, **72**, 597 (1939).

[234] Windaus and Buchholz, *Ann.*, **256**, 273 (1938).

[235] Yoder, *J. Biol. Chem.*, **116**, 71 (1936); Eck and Thomas, *ibid.*, **119**, 621, 631 (1937); Kawasaki, *J. Pharm. Soc. Japan*, **59**, 418 (1939) [*C. A.*, **34**, 1028 (1940)].

vary somewhat with the source of the acid. These bile acids are excreted in conjugation either with glycine or with taurine. In Herbivora the glycocholates predominate, while in Carnivora the taurocholates are more abundant; but the nature of the conjugation depends more on species than on diet.[236]

The derived bile acids obtained by degrading various steroids are derivatives either of cholanic acid or of its C_5 stereoisomer, *allo*cholanic acid (II). The properties and sources of the important natural and derived bile acids are listed in Table II. Of these, cholic, desoxycholic, and α-hyodesoxycholic acids are the natural bile acids that are readily available.

The bile acids are products of the synthetic activity of the liver and may originate, in part, from amino acids.[237] Relatively large quantities of these acids are formed in the liver; a dog of average size, for example, produces about 1.5 g. per day. Conjugation with glycine or with taurine appears to be a detoxication mechanism.[238]

I. Cholanic acid   II. *Allo*cholanic acid
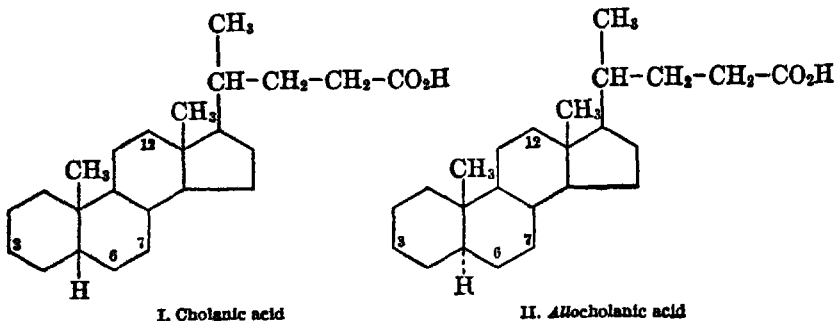
Isolation. In the bile some unconjugated bile acids probably are present, but the conjugated compounds predominate. The latter may be isolated in an impure state by salting out, or dried bile may be crystallized from absolute alcohol. After the peptide linkage is hydrolyzed with alkali, the free bile acids are isolated by crystallization from organic solvents, or a separation may be effected by extracting an ethereal solution of the mixed bile acids with various concentrations of hydrochloric acid. Cholic acid (3,7,12-trihydroxy) is extracted from an ethereal solution by

[236] Shimizu,"Über die Chemie und Physiologie der Gallensäuren," Muramoto, Okayama (1935), p. 47.

[237] Whipple and co-workers, *J. Biol. Chem.*, **80**, 658, 671, 685 (1928); **89**, 689, 705 (1930); Thannhauser and co-workers, *Arch. exptl. Path. Pharmakol.*, **130**, 292, 308 (1928); Jenke, *ibid.*, **163**, 175 (1932); Schindel, *ibid.*, **166**, 36 (1932); Schoenheimer, Rittenberg, Berg, and Rousselot, *J. Biol. Chem.*, **115**, 635 (1936).

[238] Horrall, *Physiol. Rev.*, **11**, 122 (1931); Strain and Marsh, *Am. J. Physiol.*, **115**, 82 (1936).

TABLE II

NATURAL AND DERIVED BILE ACIDS *

Bile Acid †	Position of Hydroxyl Groups	M.P. ° C.	[α]D (Alcohol)	Bile Source ‡ or Derivation
Cholanic Acids, $C_{24}H_{40}O_2$				
*Allo*cholanic		170	+22 2°§	α-Hyodesoxycholic
Bufocholanic		236	−20.6	Bufodesoxycholic
Cholanic		164	+21 6	Cholic, desoxycholic, etc.
Monohydroxy Acids, $C_{24}H_{40}O_3$				
3-Hydroxy*allo*cholanic	3(α)	208–210	+25.4	Hyodesoxycoolic
β-3-Hydroxy*allo*cholanic	3(β)	218	+17.2	Hyodesoxycholic, dihydro-cholesterol
6-Hydroxy*allo*cholanic	6	228		Hyodesoxycholic
Lithocholic	3(α)	185–186	+32.7	Man, ox, cholic
β-3-Hydroxycholanic	3(β)	176–177	+25 3	Lithocholic, coprosterol
7-Hydroxycholanic	7	96–102		7,12-Dihydroxycholanic
12-Hydroxycholanic	12	90–95		7,12-Dihydroxycholanic
Isolithocholic	(?)	185	+94 3	Goose (T), hen (T)
Dihydroxy Acids, $C_{24}H_{40}O_4$				
Bufodesoxycholic	3, (?)	197	+37	Toad (T)
Chenodesoxycholic (anthropo, gallo-)	3(α), 7(α)	140	+11.1	Goose (T), hen, man, ox, cholic
Desoxycholic	3(α), 12	176 5	+55	Man (G and T), ox (G), sheep, dog, goat (G and T), rabbit (G), deer, cholic
7,12-Dihydroxycholanic	7(α), 12	226–227		Cholic
β-7,12-Dihydroxycholanic	7(β), 12	208		Cholic
α-Hyodesoxycholic	3(α), 6	196	+ 8.4	Pig (G), hippopotamus
β-Hyodesoxycholic	3(β), 6	189–190	+ 5.1	Pig
*Allo*hyodesoxycholic	3, 6	274		α-Hyodesoxycholic
α-Lagodesoxycholic	3(α), 12	156–157	+80 4	Rabbit (G)
β-Lagodesoxycholic	(?), (?)	213	+37.4	Rabbit (G)
Ursodesoxycholic	3(α), 7(β)	203	+57 07	Polar bear (T)
Trihydroxy Acids, $C_{24}H_{40}O_5$				
Cholic (cholalic)	3(α), 7(α), 12	196–198	+35	Man (G and T), ox (G and T), sheep, dog (T), goat, snake (T), fish (T) (Apparently present in most species.)
Nutriacholic	(?), (?), (?)	198		Otter (G)
β-Phocaecholic	3, 7, 23	222–232	+27.3	Walrus (T), seal (T)
Tetrahydroxy Acids, $C_{24}H_{40}O_6$				
3,7,8,12-Tetrahydroxycholanic	3, 7, 8, 12	223–225		Rabbit (?)

* Data largely from Sobotka, "The Chemistry of the Sterids," Williams and Wilkins, Baltimore (1938).
† The configuration of rings A/B is *cis* in cholanic acid and its derivatives, *trans* in the *allo*cholanic acids, and undetermined in the "bufo" acids.
‡ The letters in parentheses indicate the type of conjugation (G = glycine, T = taurine) in the different species.
§ In chloroform.

15 per cent hydrochloric acid; the dihydroxy acids, desoxycholic and chenodesoxycholic, are removed by 25 per cent hydrochloric acid, and the monohydroxylithocholic acid is scarcely extracted at all by concentrated hydrochloric acid. Using this extraction procedure, Wieland [239] has been able to obtain from 10 liters of winter beef bile 439.9 g. of cholic, 81.3 g. of desoxycholic, and 81.2 g. of chenodesoxycholic acids. The acids that are present in small amounts in the various biles are extremely difficult to isolate and, for the most part, have not been investigated.

Nomenclature. Many of the names commonly used for the bile acids contain a prefix that shows the source of the acid. The application of this terminology is apparent from Table II. Systematically the acids are named as derivatives of cholanic acid or its stereoisomer, *allo*cholanic acid, but no system has been developed for the degradation products formed by oxidation. These compounds must be referred to by the colorful names which were given them when their structures were but partially known. Certain general rules are followed, however. The tricarboxylic or ketotricarboxylic acids formed by opening ring A at C_3—C_4 are known as bilianic acids; the several bilianic acids are differentiated by means of prefixes describing the parent compounds; thus lithocholic acid gives lithobilianic acid (p. 1361). Opening of ring A at C_2—C_3 leads to the formation of isobilianic acids. The name thilobilianic acids is applied to the tricarboxylic acids obtained by oxidative cleavage of ring B. The etiobilianic acids are formed by opening ring D after the C_{17} side chain has been completely removed (*cf.* p. 1361). The names of several other types are given in the preceding and subsequent sections.

The Nuclear Hydroxyl Groups. The bile acids may be hydroxylated in the nucleus at positions C_3, C_6, C_7, and C_{12}, or in the side chain at C_{23}. Apparently, all the natural bile acids have an hydroxyl group at C_3, and usually this is in the α-configuration. From hog bile, however, α- and β-hyodesoxycholic (3,6-dihydroxycholanic) acids have been isolated, and these appear to be epimeric about C_3.[240] There may be a further example of C_3—OH epimerism in the α- and β-lagodesoxycholic acids (p. 1424), but the large difference in the specific rotations of the two makes this seem improbable.[241] Similarly, the monohydroxy acids, lithocholic and isolithocholic, may be epimeric at C_3, but here the values for the specific rotation suggest that they are position rather than space isomers (*cf.* Structure and Optical Rotation, p. 1378). The spatial configuration of the C_3—OH has been established by the oxidative degradation of *epi*-coprosterol to lithocholic acid;[242] by the changes involved in the formation

[239] Wieland and Siebert, *Z. physiol. Chem.*, **262**, 1 (1939).
[240] Kimura, *ibid.*, **248**, 280 (1937).
[241] Kishi, *ibid.*, **238**, 210 (1936).
[242] Ruzicka and Goldberg, *Helv. Chim. Acta*, **18**, 668 (1935).

of lithocholic acid from $3(\beta)$-hydroxy-Δ^5-cholenic acid, a product of the oxidative degradation of dibromocholesterol;[242] by the conversion of lithocholic acid to a number of degradation products of *epi*coprosterol;[244] and by the fact that the esters of lithocholic acid do not form insoluble digitonides, while the esters of *epi*lithocholic acids do.[245] As is shown later, the principal bile acids may be converted to lithocholic acid, and thus the configuration that obtains in lithocholic applies to the other acids.

The steric position of the C_7—OH is not the same in all the bile acids. From the work of Lettré (p. 1378), the C_7—OH of cholic and of chenodesoxycholic acids is known to be in an α-configuration, or *trans* to the C_{10}—CH_3. Ursodesoxycholic acid, the characteristic bile acid of the polar bear, is stereoisomeric at C_7 with chenodesoxycholic acid.[246] In both α- and β-hyodesoxycholic acids, the C_6—OH is assumed to have an α-configuration.[247] In several of the bile acids an hydroxyl group is present at C_{12}. This hydroxyl group probably occupies an α- (or *trans*) configuration with respect to the adjoining C_{13}—CH_3, since catalytic hydrogenation in acetic acid (neutral medium) of dehydrocholic (3,7,12-triketocholanic) acid, or of dehydrodesoxycholic (3,12-diketocholanic) acid, returns the original bile acid.[248]

There are marked differences in the reactivity of the several nuclear hydroxyl groups. On the basis of the ease of esterification and of dehydrogenation, Wieland[249] suggested that the reactivity of the hydroxyl-hydrogen is in the order $C_7 > C_3 > C_{12}$, and that an hydroxyl group at C_6 is more reactive than one at C_3. Later work, however, shows that cold chromic acid oxidation converts hydroxyl groups at C_7 and C_{12} to carbonyl without acting on the C_3—OH.[250] Deacetylation of acetyl compounds proceeds more readily at C_3 than at C_7.[251]

Similar differences in reactivity are found in the dehydro(keto)-cholanic acids. Catalytic hydrogenation of the carbonyl group proceeds most easily at C_3, with intermediate ease at C_6 and C_7, and with difficulty at C_{12}. With Clemmensen reduction only the C_3 carbonyl can be

[242] Schoenheimer and Berliner, *J. Biol. Chem.*, **115**, 19 (1936)

[244] Reindel and Niederländer, *Ann.*, **522**, 218 (1936).

[245] Reindel and Niederländer, *Ber.*, **68**, 1243 (1935).

[246] Iwasaki, *Z. physiol. Chem.*, **244**, 181 (1936); Miyaji, *ibid.*, **250**, 31 (1937); Miyaji and Isaka, *J. Biochem. (Japan)*, **30**, 297 (1939) [*Chem. Zentr.*, (I) 2165 (1940)].

[247] Tukamoto, *J. Biochem. (Japan)*, **32**, 451, 461 (1940).

[248] Borsche and Feske, *Z. physiol. Chem.*, **176**, 109 (1928).

[249] Wieland and Dane, *ibid.*, **210**, 268 (1932); Wieland, Dane, and Martins, *ibid.*, **215**, 15 (1933).

[250] Kasiro and Shimada, *ibid.*, **249**, 220 (1937); Bergstrom and Haslewood, *J. Chem. Soc.*, 540 (1939).

[251] Wieland and Kapitel, *Z. physiol. Chem.*, **212**, 269 (1932).

reduced,[223] while with Wolff-Kishner reduction a C_3 carbonyl is abnormally converted to hydroxyl unless an excess of hydrazine is used.[125]

The characteristic bitter taste of bile is due to the bile acids. This taste is a function of the degree of hydroxylation, for the mono- and dihydroxy acids are tasteless. Conjugation with glycine or taurine enhances and modifies the taste considerably, producing in many instances an initial sweet taste which is rapidly replaced by bitter.

The Unsaturated Bile Acids. When the bile acids are heated *in vacuo*, dehydration occurs with the production of mixtures of isomeric unsaturated acids.[253] The physical properties of certain of these unsaturated acids are shown in Table III. The course of the dehydration is

TABLE III

THE UNSATURATED BILE ACIDS

Acid	Formula	M.P. °C.	$[\alpha]_D$ (Alcohol)
α-Lithocholenic (Δ^2 ?)............	$C_{24}H_{38}O_2$	156	+16.3°
β-Lithocholenic (Δ^3 ?)........	$C_{24}H_{38}O_2$	160	+18.7
Δ^5-Cholenic (?)......................... ...	$C_{24}H_{38}O_2$	160	−66.8
3(β)-Hydroxy-Δ^5-cholenic................ ...	$C_{24}H_{38}O_3$	241–242	
Apocholic (3,12-dihydroxy-$\Delta^{8.14}$?)........ ...	$C_{24}H_{38}O_4$	173–174	+45.5
Dihydroxycholenic (3,12-dihydroxy-$\Delta^{14\ 15}$?) ...	$C_{24}H_{38}O_4$	255–256	+57.6
Isodihydroxycholenic.......................	$C_{24}H_{38}O_4$	198	+ 5.9
3(β)-Hydroxy-$\Delta^{5,7}$-choladienic	$C_{24}H_{36}O_3$	214–216	−69
α-Dihydroxycholadienic ($\Delta^{7.8-14.15}$?)...... . .	$C_{24}H_{36}O_4$	252–255	−35.5
β-Dihydroxycholadienic ($\Delta^{8.9-14:15}$?)....... ...	$C_{24}H_{36}O_4$	253–255	+71

illustrated by the products formed from lithocholic [3(α)-hydroxycholanic] acid.[254] The mixture obtained on dehydration contains about nine parts of an α-acid and one part of a higher-melting β-acid. The lower-melting α-lithocholenic acid is probably unsaturated at $C_2 : C_3$, and the β-lithocholenic acid at $C_3 : C_4$. Separation of the mixture is effected by bromination and crystallization of the dibromides. In this way, three dibromides are obtained, one melting at 171°, another at 233°, and the third at 240°. The two melting at 171° and 233°, respectively, give α-lithocholenic acid when debrominated with zinc; these two dibromides are probably epimers in which the bromine is *cis, cis* in one, and *trans, cis* in the other.

[253] Kawai, *ibid.*, **214**, 71 (1933).
[253] Shimizu, Oda, and Makino, *ibid.*, **213**, 136 (1932).
[254] Wieland, Kraus, Keller, and Ottawa, *ibid.*, **241**, 47 (1936).

The removal of water from ring B takes place largely through the splitting off of an hydroxyl with an adjacent tertiary hydrogen. An example of this is the dehydration of 6-hydroxy*allo*cholanic acid to a *levorotatory* compound, which probably is unsaturated at $C_5 : C_6$. In ring C with the hydroxyl group at C_{12}, dehydration can give but one product, as C_{13} is quaternary.

Acid dehydrating agents, such as zinc chloride and sulfuric acid, remove water from ring B in cholic and chenodesoxycholic acids. In the case of cholic acid, the principal product is apocholic acid, which appears to have the structure 3,12-dihydroxy-$\Delta^{8(14)}$-cholenic acid. In smaller yield there is obtained a dihydroxycholenic acid, which may be unsaturated at $C_{14} : C_{15}$.[255] It can be hydrogenated to desoxycholic acid, but apocholic acid cannot be reduced catalytically. Treatment of apocholic acid with hydrochloric acid partially rearranges it to an isodihydroxycholenic acid, which is also obtainable from cholic acid by the action of hydrogen chloride; isodihydroxycholenic acid cannot be reduced catalytically. Evidently the isomers result from a rearrangement of a double bond about C_8, or from C_8 to a neighboring carbon atom. In a general way, there appears to be an analogy between these compounds and the α-stenols (p. 1387).

Bromine dehydrogenates apocholic acid to a mixture of two isomeric acids, α- and β-dihydroxycholadienic acids.[256] The mechanism is addition of bromine followed by spontaneous loss of hydrogen bromide. The two acids may be produced from apocholic acid by the action of perbenzoic acid to give the α-dienic, or selenium dioxide to form the β-dienic acid.[257] Both these dienic acids can be partially hydrogenated, the α-dienic acid yielding apocholic acid, and the β-dienic acid an isomeric β-apocholic acid which may be stereoisomeric about C_9. Isodihydroxycholenic acid, when dehydrogenated with bromine, gives a dienic acid differing from the known isomers, and hydrogenation of the dienic acid likewise gives an unidentifiable product.[258]

Bile acid analogs of the 7-dehydrosterols have not been investigated for pinacol formation and for phototransformations, although 3(β)-hydroxy-$\Delta^{5,7}$-choladienic acid has been prepared from 3(β)-hydroxy-Δ^5-cholenic acid.[259] It is known, however, that irradiation of apocholic acid

[255] Boedeker, *Ber.*, **53**, 1852 (1920); Boedeker and Volk, *Ber.*, **54**, 2489 (1921); Borsche and Todd, *Z. physiol. Chem.*, **197**, 173 (1931); Wieland and Dane, *ibid.*, **212**, 263 (1932); Yamasaki, *ibid.*, **220**, 42 (1933); **233**, 10 (1935); Yamasaki and Takahashi, *ibid.*, **256**, 21 (1938).

[256] Wieland and Deulofeu, *Z. physiol. Chem.*, **196**, 127 (1931).

[257] Callow, *J. Chem. Soc.*, 462 (1936).

[258] Wieland, Dietz, and Ottawa, *Z. physiol. Chem.*, **244**, 194 (1936).

[259] Haslewood, *J. Chem. Soc.*, 224 (1938); *cf.* Dane and Wulle, *Z. physiol. Chem.*, **257**, 1 (1940).

or of dioxycholenic acid without a sensitizer produces an equilibrium mixture of the two acids.[260]

The Color Reactions of the Bile Acids. The unsaturated acids are probably intermediates in the color reactions given by the bile acids. In the *Pettenkofer reaction*, or its modifications, an aqueous solution of the bile acid is treated with concentrated sulfuric acid in the presence of sugars,[261] furfuraldehyde,[262] or other aldehyde.[263] The violet color produced by cholic and apocholic acid, when dissolved in 25 per cent hydrochloric acid, is known as the *Hammarsten reaction*.[264] The color produced by the addition of solid bile acids to concentrated sulfuric acid is the *Liebermann reaction*.[265] As, with the color reactions of the sterols, color production is probably due to the formation of halochromic salts.

Transformations of the Nucleus. The nuclear hydroxyl groups weaken the several rings at the point of attachment, and, by the proper selection of oxidizing agent, stepwise degradation may be effected. Cold chromic acid produces the least change, converting the carbinol groups to carbonyl to form the dehydro acids,[266] while concentrated nitric acid and potassium permanganate open the rings. The possibilities of such transformations are illustrated in the degradation of desoxybilianic acid (p. 1363). Concentrated nitric acid acting over a period of time on this acid not only opens ring C, but also probably oxidizes the C_{10} methyl group to carboxyl, forming chollepidanic acid (Gr., *lepis* = scale) (III).[267] Less vigorous treatment with nitric acid merely opens ring C to produce choloidanic acid (IV).[268] Pyrocholoidanic acid (V), the pyrolytic product from IV, on oxidation first with permanganate and then with nitric acid, passes through the stage of prosolanellic acid (VI) to solanellic acid (*solus anellus* = one ring) (VII);[269] pyrosolanellic acid (VIII) in turn yields biloidanic acid (IX).[270] The last may be produced more easily by the action of mixed acids on bilianic acid (XI),[271] the product of partial nitric acid oxidation of dehydrocholic acid (X).

[260] Sihn, *Z. physiol. Chem.*, **257**, 232 (1938).

[261] Pettenkofer, *Ann.*, **52**, 90 (1844).

[262] Mylius, *Z. physiol. Chem.*, **11**, 492 (1887); Gregory and Pascoe, *J. Biol. Chem.*, **83**, 30 (1929); Reinhold and Wilson, *ibid.*, **96**, 637 (1932).

[263] Woker and Antener, *Helv. Chim. Acta*, **27**, 1345 (1938); Kaziro and Shimada, *Z. physiol. Chem.*, **254**, 57 (1938).

[264] Hammarsten, *Z. physiol. Chem.*, **61**, 495 (1909); Yamasaki, *ibid.*, **220**, 42 (1933).

[265] *Cf.* Dana, *Tabulae Biologicae Periodicae*, III, 58 (1933), for tabulation of color changes.

[266] Hammarsten, *Ber.*, **14**, 71 (1881).

[267] Wieland and Kraft, *Z. physiol. Chem.*, **211**, 203 (1932).

[268] Wieland and Kulenkampff, *ibid.*, **108**, 306 (1920).

[269] Wieland and Schulenburg, *ibid.*, **114**, 167 (1921).

[270] Schenck, *ibid.*, **110**, 167 (1920); **112**, 38 (1921).

[271] Wieland and Schlichting, *ibid.*, **119**, 76 (1922).

$C_4H_8CO_2H$

HO_2CCH_3
HO_2C
CH_3

HO_2C
HO_2C

← HNO₃ — Desoxybilianic acid — HNO₃ →

IV. Choloidanic acid

$C_4H_8CO_3H$

HO_2CCH_3
HO_2C
HO_2C

HO_2C
HO_3C

III. Chollepidanic acid

| Thermal decomposition

$C_4H_8CO_2H$

HO_2CCH_3
HO_3C
CH_3

O=

KMnO₄ →

HO_3C

V. Pyrocholoidanic acid

$C_4H_8CO_2H$

HO_2CCH_3
HO_2C
CH_2

HO_3C O=

VI. Prosolanellic acid

HNO₃ →

$C_4H_8CO_2H$

HO_2CCH_3
HO_2C
CH_3

HO_2C
HO_2C HO_2C

Pyrolysis →

VII. Solanellic acid

$C_4H_8CO_2H$

HO_2CCH_3
HO_2C
CH_3

HO_2C O=

VIII. Pyrosolanellic acid

HNO₃ →

$C_4H_8CO_2H$

HO_2CCH_3
HO_2C
CH_3

HO_2C
HO_2C CO_2H

IX. Biloidanic acid

HNO₃+H₂SO₄ ↗

O $C_4H_8CO_2H$
CH_3

HNO₃ →

CH_3

HO_2C
HO_2C

O=

XI. Bilianic acid

KMnO₄ →

[CH_3

HO_2C
HO_3C

O=
O]

XII. Triketo acid
$C_{24}H_{32}O_9$

Benzilic acid rearrangement ↘

O $C_4H_8CO_2H$
CH_3

CH_3

O=
O=

X. Dehydrocholic acid

O $C_4H_8CO_2H$
CH_3

HO_2C
HO_2C
OH
CO_2H

XIII. Cilianic acid

Bilianic acid when oxidized with permanganate suffers oxidation in ring B to give a triketo acid (XII) which, when treated with acid, undergoes a benzilic acid rearrangement to form cilianic acid (XIII).[272]

[272] Schenck, ibid., **37**, 59 (1913); **242**, 81 (1936); **244**, 245 (1936).

Chromic acid oxidation in the cold of α-hyodesoxycholic acid gives the dehydro acid, 3,6-diketocholanic acid, which is readily isomerized to the *allo* series by warming with strong acids or alkalies.[273] The resulting 3,6-diketo*allo*cholanic (β-dehydrohyo) acid serves as a source of a variety of *allo* derivatives. α-Hyodesoxycholic acid (XIV) is converted by the action of hypobromite to an hydroxytricarboxylic acid (XV), which may be reduced by the action of hydrogen iodide to lithobilianic acid. Treatment of the hydroxytricarboxylic acid with sodium ethoxide dehydrates it to lithobilienic acid, and this acid is converted by catalytic hydrogenation to *allo*lithobilianic acid (XVI). The isomeric *allo*-isolithobilianic acid (XIX) is obtained from 3,6-diketo*allo*cholanic acid (XVII) by permanganate or hypobromite oxidation to a 6-ketotricarboxylic acid (XVIII), which is then reduced by the Wolff-Kishner method to the desired *allo* acid. As has been cited previously, these isomeric bilianic acids played an important part in the establishment of the spatial configuration about C_5 (p. 1369).

XIV. α-Hyodesoxycholic acid

XV. Hydroxytricarboxylic acid

XVI. *Allo*lithobilianic acid

Cold CrO₃ oxidation. and rearrangement

XVII. 3,6-Diketo*allo*cholanic acid

XVIII. Keto-tricarboxylic acid

XIX. *Allo*isolithobilianic acid
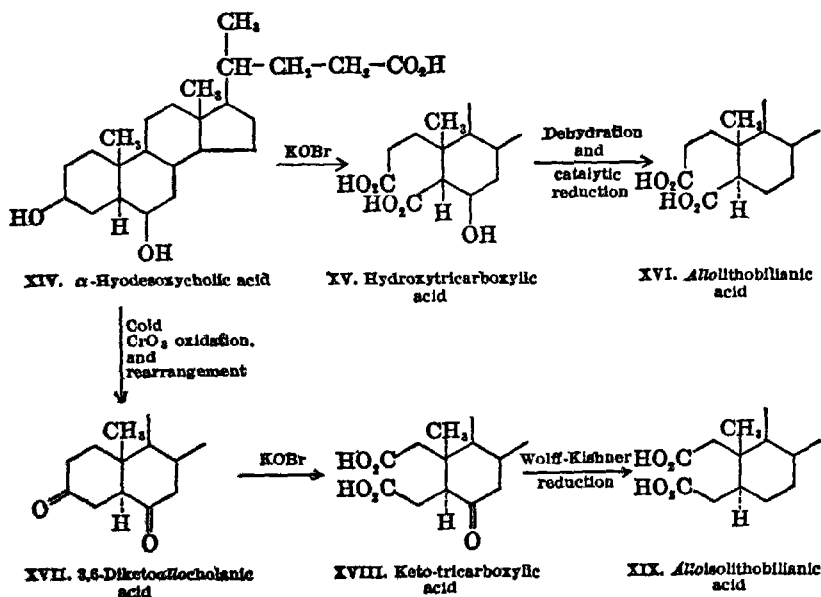
Bufocholanic Acid. From the bile of the toad, the specific dihydroxy bile acid, bufodesoxycholic, has been isolated;[274] it differs stereochemically from the others. The corresponding dehydro acid can be rear-

[273] Windaus and Bohne, *Ann.*, **433**, 278 (1923); Windaus, *Ann.*, **447**, 233 (1926); Shibuya and Miki, *Z. physiol. Chem.*, **206**, 279 (1932).

[274] Okamura, *J. Biochem. (Japan)*, **8**, 351 (1928); **10**, 5 (1928); **11**, 103 (1929).

ranged like dehydrohyodesoxycholic acid, but the bufocholanic acid produced by Clemmensen reduction of these two dehydro acids is not identical with either cholanic or *allo*cholanic acid. On opening ring A of these bufodehydro acids by the action of hypobromite, ketotricarboxylic acids are produced, which, on Clemmensen reduction, are converted to *allo*lithobilianic acid. From this evidence, one of the hydroxyl groups is placed at C_3, but the position of the other hydroxyl is uncertain. Obviously this second hydroxyl group is adjacent to one of the centers of asymmetry.

The C_{10}—CH_3 Group. With many of the bile acids, pyrolysis in an atmosphere of carbon dioxide splits off the C_{10}—CH_3 as methane. The reaction parallels the conversion of the sterol pinacols to the norsterols. The lability of the methyl group is markedly affected by structure; for example, in apocholic acid, more than a third of the molecule suffers this type of change, but with most of the bile acids very little methane is split off.[275]

Molecular Compounds. The property of forming molecular compounds is pronounced in the bile acids, and, as commonly obtained, they crystallize with a molecule of solvent of crystallization. One of the unique molecular compounds is the blue compound $(C_{24}H_{40}O_5 \cdot I)_4 \cdot KI \cdot H_2O$, formed when an alcoholic solution of cholic acid and a solution of iodine in potassium iodide are mixed in the correct proportions.[276]

With any of a wide variety of substances, desoxycholic acid forms a series of water-soluble molecular compounds which are collectively known as the *choleic acids*. The compounds formed with the fatty acids were the first examples noted and have since received considerable study.[277] With increasing molecular weight, the molecular ratio of bile acid : fatty acid changes from 1 : 1 with acetic acid to 8 : 1 with stearic acid. The variations are shown graphically in Fig. 1 both for fatty acids and dicarboxylic acids. Since formation of choleic acids is due to coördinate valences, the compounds are conveniently described by a coördination number which expresses the number of molecules of desoxycholic acid combined with one molecule of a second substance. As the curves show, the ratio changes by steps. The significance of this is a matter of speculation, but usually there is assumed to be a packing of molecules along the lower edge of the nucleus and the side chain (*cf.* structure I)

[275] Wieland and Dane, Z. physiol. Chem., **212**, 263 (1932).

[276] Mylius, ibid., **11**, 306 (1887); Küster, Z. physik. Chem., **16**, 156 (1895); Barger and Field, J. Chem. Soc., **101**, 1404 (1912).

[277] Wieland and Sorge, Z. physiol. Chem., **97**, 1 (1916); Rheinboldt and co-workers, Ann., **451**, 256 (1927); Z. physiol. Chem., **180**, 180 (1928); Ann., **473**, 249 (1929); J. prakt. Chem., **153**, 313 (1939); Sobotka and Goldberg, Biochem. J., **26**, 555 (1932).

caused by the "enhancing" effect of the hydroxyl group at C_{12}. Supporting evidence for this theory is furnished by the fact that apocholic acid forms coördination compounds comparable to those of desoxycholic acid, while chenodesoxycholic and cholic acids do not. The choleic acids formed from enolizable ketones exhibit the peculiarity of being combined completely in the enolized form.[278] Since functional groups are not necessary for the formation of choleic acids, these compounds have been
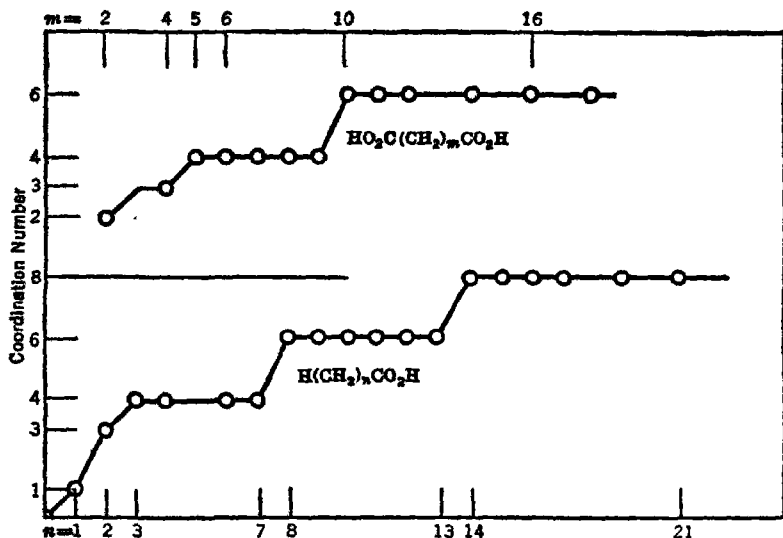
Fig. 1.—Relation between length of aliphatic chain and coördination number of the fatty acid choleic acids. *

suggested as a means of resolving racemic mixtures of optically active but inert compounds, such as hydrocarbons.[278]

The soluble salts of the bile acids lower the surface tension of water to a marked degree.[279] This property may be associated with the phenanthrene structure, for other compounds containing a phenanthrene nucleus, such as the saponins (p. 1454) and abietic acid, exhibit the same behavior.

Natural Bile Acids and Their Derivatives. Cholic acid, the most common bile acid, is found in the bile of many species (cf. Table II). In beef bile, which has been investigated most extensively, cholic acid is accompanied by smaller amounts of desoxycholic and chenodesoxycholic

[278] Sobotka and Goldberg, Biochem. J., 26, 905 (1932); Marx and Sobotka, J. Org. Chem., 1, 275 (1936).

* From Sobotka, Chem. Rev., 15, 362 (1934). (Courtesy of the publishers.)

[279] Allen, J. Biol. Chem., 22, 505 (1915).

acids together with traces of lithocholic, sterocholic, and Weyland's acids.[280] The rare lithocholic acid was first isolated from cattle gallstones,[281] and apparently these and hog gallstones [282] are the only satisfactory natural sources. Because of the inaccessibility of many biles, a considerable amount of work has been directed towards the conversion of the common to the uncommon bile acids. In addition to the preparation of lithocholic [283] and chenodesoxycholic [284] acids, a number of isomeric, monohydroxy acids have been obtained. Those reported are *allolitho*cholic [285] [3(α)-hydroxy*allo*cholanic], 3(β)-hydroxy*allo*cholanic,[286] 6-hydroxy*allo*cholanic,[285] 7-hydroxycholanic,[243] and 12-hydroxycholanic [287] acids. When dihydroxycholenic acid is oxidized with dilute permanganate, the tetrahydroxy acid, 3,7,8,12-tetrahydroxycholanic acid, results.[288] This acid may be identical with an acid isolated from rabbit bile.[289] Barbier-Wieland degradation has been applied to desoxycholic,[290] chenodesoxycholic, [291] and hyodesoxycholic [292] acids as well as to cholic acid (p. 1360).

In the course of the oxidative degradation of the saturated sterols for the manufacture of sex hormones, a number of bile acid derivatives have become available, particularly 3(β)-hydroxy-Δ⁵-cholenic acid. The physical characteristics and the literature of some of the other degradation products have been summarized by Reindel.[244]

Miscellaneous Bile Acids. In addition to the acids mentioned above, a number have been isolated from various sources. Wieland [280] has isolated from beef bile a molecular compound of chenodesoxycholic and 3-hydroxyketocholanic acids; the molecular compound is known as Weyland's acid. A keto acid, 3-hydroxy-6-keto*allo*cholanic acid, has

[280] Wieland and Jacobi, *Z. physiol. Chem.*, **148**, 232 (1925); Wieland and Kishi, *ibid.*, **214**, 47 (1933).

[281] Fischer, *ibid.*, **73**, 204 (1911).

[282] Schoenheimer and Johnston, *J. Biol. Chem.*, **120**, 499 (1937); Schenck, *Z. physiol. Chem.*, **256**, 159 (1938).

[283] Borsche and Hallwass, *Ber.*, **55**, 3318 (1922); Wieland, Dane, and Scholz, *Z. physiol. Chem.*, **211**, 266 (1932); Marker and Lawson, *J. Am. Chem. Soc.*, **60**, 1334 (1938); Dutcher and Wintersteiner, *ibid.*, **61**, 1992 (1939); Bergström and Haslewood, *J. Chem. Soc.*, **540** (1939).

[284] Kawai, *Z. physiol. Chem.*, **214**, 71 (1933); Miyaji, *ibid.*, **250**, 31 (1937).

[285] Wieland and Dane, *ibid.*, **212**, 41 (1932).

[286] Wieland and Dane, *ibid.*, **210**, 268 (1932).

[287] Wieland and Schlichting, *ibid.*, **150**, 267 (1925).

[288] Wieland and Dane, *ibid.*, **206**, 243 (1932).

[289] Windaus and van Schoor, *ibid.*, **173**, 312 (1928).

[290] Hoehn and Mason, *J. Am. Chem. Soc.*, **60**, 1493 (1938); Kasuno and Shimizu, *J. Biochem. (Japan)*, **29**, 421 (1939) [*Chem. Zentr.*, (II) 2791 (1939)].

[291] Ishihara, *J. Biochem. (Japan)*, **27**, 265 (1938) [*Chem. Zentr.*, (I) 2315 (1940)].

[292] Kimura and Sugiyama, *ibid.*, **29**, 409 (1939) [*Chem. Zentr.*, (II) 2792 (1939)]; Marker and Krueger, *J. Am. Chem. Soc.*, **62**, 79 (1940).

been obtained from pig bile.[283] In rabbit bile, desoxycholic acid is predominantly present, but there are also small amounts of the isomeric acids, α- and β-lagodesoxycholic acids; the α-acid is epimeric at C_{12} with desoxycholic acid, but the structure of the β-acid is unknown.[241]

Two C_{28} bile acids have been isolated. The first, sterocholic acid, $C_{28}H_{46}O_4$, was obtained from ox bile,[280] and related compounds are present in the bile of the snapping turtle.[294] The second C_{28} acid, trihydroxysterocholenic acid (XX), $C_{28}H_{46}O_5$, was isolated from toad bile.[295] The similarity of the side chain of this acid to that of ergosterol is striking. Related to this toad bile acid is tetrahydroxynorsterocholanic acid
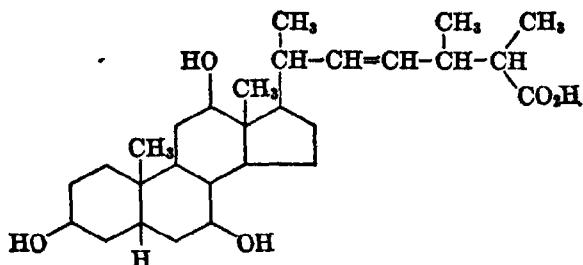
XX. Trihydroxybufosterocholenic acid
(Steric position of hydroxyl groups uncertain)
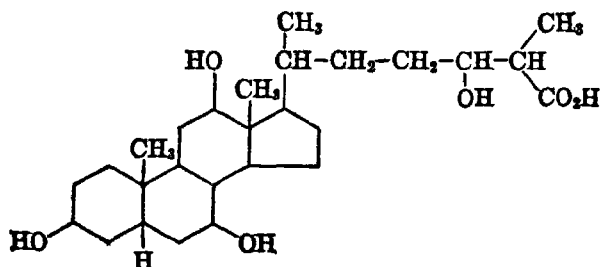
XXI. Tetrahydroxynorsterocholanic acid

(XXI), which has been isolated from the bile of the "Gigi" fish.[296] The ring hydroxyl groups of this latter acid have been placed by degradations to hyodesoxycholic acid and to 12-ketocholanic acid.

One bile acid with nuclear unsaturation has been isolated from natural sources. This has been obtained from chicken bile, and it appears to have the structure 3-hydroxy-$\Delta^{8(14)}$-cholenic acid.[297]

[283] Fernholz, Z. physiol. Chem., **232**, 202 (1935); Schoenheimer and Johnston, reference 282.

[294] Yamasaki and Fujii, Z. physiol. Chem., **244**, 173 (1936); Kim, J. Biochem. (Japan), **30**, 247 (1939) [C. A. **34**, 1327 (1940)].

[295] Shimizu and Oda, Z. physiol. Chem., **227**, 74 (1934); Shimizu and Kazuno, ibid., **233**, 67, 74 (1935); ibid., **244**, 167 (1936).

[296] Ohta, ibid., **258**, 53 (1939); Isaka, ibid., **266**, 177 (1940).

[297] Takahashi, ibid., **255**, 277 (1938).

The Bile Alcohols. From the bile of the shark, of frogs, and of toads, a number of polyhydric steroid alcohols have been isolated, usually as the sodium salts of the sulfuric acid esters. In the shark the salt of the sulfuric acid ester of scymnol (XXII), $C_{27}H_{48}O_5$, is present.[298] All the structural details of this compound have been determined except for one secondary hydroxyl group. As structure XXII shows, scymnol is one of
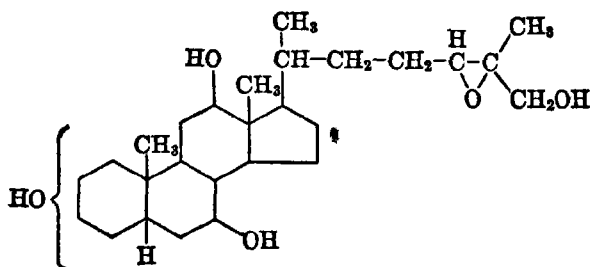
XXII. Scymnol
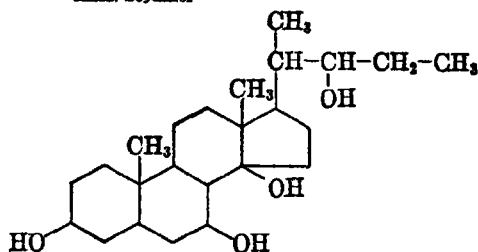
XXIII. Tetrahydroxycholane
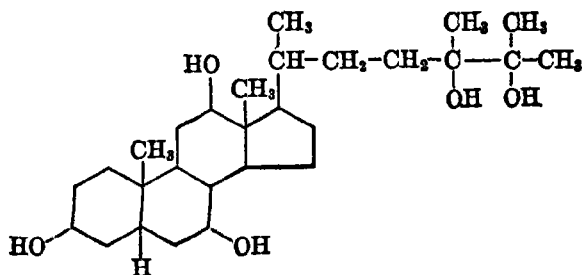
XXIV. Pentahydroxybufostane

the few steroids in which a C_3—OH group is not present. Allied with scymnol are the compounds tetrahydroxycholane (XXIII), present as a C_{14} sulfuric acid ester in frog bile,[299] and pentahydroxybufostane (XXIV), found in small quantities in toad bile.[300] The structures of both

[298] Hammarsten, *ibid.*, **24**, 323 (1898); Windaus, Bergmann, and König, *ibid.*, **189**, 148 (1930); Tschesche, *ibid.*, **203**, 263 (1931).

[299] Kurauti and Kasuno, *ibid.*, **262**, 53 (1939).

[300] Kasuno, *ibid.*, **266**, 11 (1940).

compounds have been determined by the standard methods of degradation. In the case of pentahydroxybufostane, chromic acid oxidation of the side chain is accompanied by a pinacol rearrangement to give the con-

figuration $-CH_2-\overset{\overset{\displaystyle O}{\displaystyle \|}}{C}-\overset{\overset{\displaystyle CH_3}{\displaystyle |}}{\underset{\underset{\displaystyle CH_3}{\displaystyle |}}{C}}-CH_3$, which is novel in the steroid group.
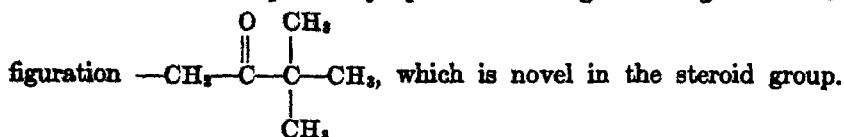
These bile alcohols may be viewed on biogenetic grounds as evolutionary stages in the formation of the bile acids.

The Conjugated Bile Acids. Some conjugated bile acids can be obtained pure by crystallization from absolute alcohol of dried bile or of the products obtained by salting out.[301] In general, however, these are not satisfactory procedures, and the pure acids are best obtained by reacting glycine or taurine with the azides of the individual bile acids [302] or with the acid chlorides of the formylated bile acids.[303] The conjugated acids are more acidic and their salts are more soluble than the free bile acids. The conjugated derivatives of desoxycholic acid will not form choleic acids.[303, 304]

Physiological Transformations of the Bile Acids. A number of Japanese workers have injected a variety of bile acids and their derivatives in various animals and, from the site and nature of the excretion products, have drawn conclusions as to the biochemical transformations brought about in the body. Injected cholic acid is eliminated in the bile of guinea pigs in small amounts as desoxycholic, chenodesoxycholic, and 3-hydroxy-7-ketocholanic acids.[305] This suggests that the body can oxidize C_7 and C_{12} hydroxyl groups to carbonyl with subsequent reduction to methylene. In accord with this, injected ketonic derivatives suffer reduction in the body with conversion of C_7 and C_{12} carbonyl groups to methylene, and of C_3 and C_6 carbonyls to hydroxyls.[306] In the case of C_3 carbonyls, the products excreted by the liver have an α-configuration; those excreted by the kidneys may have a β-configuration. When 3,6-diketocholanic acid is injected subcutaneously in toads, it is eliminated as 3(β)-hydroxy-6-ketoallocholanic acid in the urine.[307] Injected unsat-

[301] Abderhalden, "Handbuch der biologischen Arbeitsmethoden," Urban and Schwarzenberg, Berlin (1925), i, VI, 211.

[302] Bondi and Mueller, Z. physiol. Chem., 47, 4991 (1906); Cortese, J. Am. Chem. Soc., 59, 2532 (1937).

[303] Cortese and Baumann, J. Am. Chem. Soc., 57, 1393 (1935); J. Biol. Chem., 113, 779 (1936); Cortese and Bashour, ibid., 119, 117 (1937).

[304] Wieland, Z. physiol. Chem., 106, 181 (1919).

[305] Kim, ibid., 261, 97 (1939).

[306] Miyaji, ibid., 254, 104 (1938); Kim, ibid., 255, 267 (1938).

[307] Tukamoto, ibid., 260, 210 (1939).

urated bile acids appear to be hydroxylated or to suffer rearrangement.[308] This type of investigation is not entirely conclusive, however, since only fractional per cents of injected material can be accounted for.

THE CARDIAC AGLUCONS AND THE TOAD POISONS

A number of glycosides of plant origin and the nitrogenous venoms secreted by the parotid glands of toads possess valuable cardiotonic properties. The administration of these substances to individuals with damaged heart function results in a decrease in the rate and an increase in the intensity of the heart beat. Overdosage produces pernicious vomiting and stoppage of the heart in systolic standstill. The cardiac substances are used for other purposes in medicine, but the principal use is for their characteristic heart action. In addition to their therapeutic use, certain of the glycosides have been employed as arrow and ordeal poisons by savage tribes, particularly by the natives of Africa and of the Malayan peninsula.[309] Acid or enzymatic hydrolysis of the glycosides or of the venoms splits off the sugar residues or the nitrogenous bases to give the so-called genins (Fr., génie, spirit). In the case of the glycosides these hydrolytic products are also called aglucons. The free genins are sparingly soluble and are convulsive poisons rather than satisfactory heart stimulants; they are valueless medicinally.

The Cardiac Aglucons

The Cardiac Glycosides. The chief sources of the cardiac glycosides are the members of the plant orders *Apocynaceae* and *Scrophulariaceae*. Of the latter order certain genera of *Digitalis* (foxglove) furnish most of the drugs of therapeutic value. The principal glycosides, their sources, and their hydrolytic products are listed in Table IV. In this compilation the melting points have not been given, since these are functions rather of the method of purification and of the rate of heating than of the glycosides themselves. In a few instances there is some uncertainty as to the nature of the sugar portion of the molecule.

The cardiac glycosides are obtained by extraction from the plant tissues indicated in Table IV.* The isolation of the pure glycosides in quantity is difficult because of the low content in the plant tissues and because of the presence of other substances that materially modify the solubility relations. Most of the glycosides that have been studied are probably

[308] Mori, *ibid.*, **258**, 143 (1939); Sihn, *ibid.*, **261**, 93 (1939).

[309] Lewin, "Die Pfeilgifte," Barth, Leipzig (1923).

* For preparative details of the glycosides see Van Rijn, "Die Glycoside," Borntraeger, Berlin (1931); Stoll, "The Cardiac Glycosides," The Pharmaceutical Press, London (1937).

TABLE IV

THE PRINCIPAL CARDIAC GLYCOSIDES *

Glycoside	Formula	Plant Sources †	Genin	Sugars
				Hydrolytic Products
Apocynaceae				
Neriantin..........	$C_{29}H_{42}O_9 \cdot 1\frac{1}{2}H_2O$	*Nerium oleander* (L)	Neriantogenin	Glucose
Ouabain..........	$C_{29}H_{44}O_{12}$	Ouabaio tree (R); *Strophanthus gratus* (S)	Anhydroouabagenin	Rhamnose
Adynerin..........	$C_{30}H_{44}O_7$	*Nerium oleander* (L)	Adynerigenin	Oleandrose (?)
Cymarin..........	$C_{30}H_{44}O_9$	*Strophanthus kombé* (S)	Strophanthidin	Cymarose
Sarmentocymarin....	$C_{30}H_{46}O_8$	*Strophanthus sarmentosus* (S)	Sarmentogenin	Sarmentose
Oleandrin..........	$C_{32}H_{48}O_9$	*Nerium oleander* (L)	Gitoxigenin	Oleandrose (and acetic acid)
k-Strophanthin-β....	$C_{36}H_{54}O_{14}$	*Strophanthus kombé* (S)	Strophanthidin	Strophanthobiose (cymarose and glucose)
Thevetin..........	$C_{42}H_{66}O_{18}$	*Thevetia nerifolia* (S)	Anhydrothevetigenin	2 Glucose and digitalose (?)
Scrophulariaceae				
Digitoxin..........	$C_{41}H_{64}O_{13}$	*Digitalis purpurea* (L)	Digitoxigenin	3 Digitoxose
Gitoxin..........	$C_{41}H_{64}O_{14}$	*Digitalis purpurea* (L)	Gitoxigenin	3 Digitoxose
Digoxin..........	$C_{41}H_{64}O_{14}$	*Digitalis lanata* (L)	Digoxigenin	3 Digitoxose
Purpurea glycoside A..	$C_{47}H_{74}O_{18}$	*Digitalis purpurea* (L)	Digitoxigenin	3 Digitoxose and 1 glucose
Purpurea glycoside B..	$C_{47}H_{74}O_{19}$	*Digitalis purpurea* (L)	Gitoxigenin	3 Digitoxose and 1 glucose

	Formula	Aglycone	Sugars	Source
Digilanide A............	$C_{49}H_{76}O_{19}$	Digitoxigenin	2 Digitoxose, acetyldigitoxose, and glucose	*Digitalis lanata* (L)
Digilanide B............	$C_{49}H_{76}O_{20}$	Gitoxigenin	2 Digitoxose, acetyldigitoxose, and glucose	*Digitalis lanata* (L)
Digilanide C............	$C_{49}H_{76}O_{20}$	Digoxigenin	2 Digitoxose, acetyldigitoxose, and glucose	*Digitalis lanata* (L)
Asclepiadaceae				
Periplocymarin........	$C_{29}H_{46}O_{8}$	Periplogenin	Cymarose	*Periploca graeca* (W, B)
Usarin................	$C_{35}H_{54}O_{14}$	Anhydrouzarigenin	2 Glucose	Uzara Tree
Periplocin............	$C_{36}H_{56}O_{13}$	Periplogenin	Cymarose and glucose	*Periploca graeca* (B); *Ghomphocarpus*
Moraceae				
α-Antiarin	$C_{29}H_{42}O_{11}$	Antiarigenin	Antiarose	*Antiaris toxicaria* (La)
β-Antiarin	$C_{29}H_{42}O_{11}$	Anhydroantiarigenin	Rhamnose	*Antiaris toxicaria* (La)
Liliaceae				
Convallatoxin.........	$C_{29}H_{43}O_{10}$	Convallatoxigenin	Rhamnose	*Convallaria majalis* (F, L)
Proscillaridin A.......	$C_{30}H_{42}O_{8}$	Scillaridin A	Rhamnose	*Scilla maritima*
Scillaren A...........	$C_{36}H_{52}O_{13}$	Scillaridin A	Rhamnose and glucose	*Scilla maritima*

* Compiled from reviews by Elderfield and Tschesche (see end of chapter); from Neumann, *Ber.*, **70**, 1547 (1937); and from Tschesche *et al.*, *Ber.*, **71**, 654, 1927 (1938).

† Abbreviations: B = bark, F = flowers, L = leaves, La = latex, R = root, S = seeds, W = wood.

not the true plant glycosides, since there are enzymes in the plants that rapidly bring about a partial hydrolysis. The digilanides A, B, and C, the purpurea glycosides, and scillaren A are representatives of the true plant glycosides.[310] The other compounds of Table IV are obtained from the dried tissues and are probably enzymatic degradation products, as has been shown to be true in a few instances. Thus, the juices of the fresh leaves have been used to convert purpurea glycoside A to digitoxin, purpurea glycoside B to gitoxin, and digilanide C to digoxin.[310] From the dried leaves the glycosides are generally extracted by means of alcohol, the tannins removed, and the products purified by precipitation and crystallization, or by partition between solvents, e.g., chloroform and water or aqueous methanol.

As many as four sugar molecules may be present in the native glycosides, and apparently they are attached to the genin at the C_3—OH (see formula I below). The sugars are for the most part α-desoxy sugars (cymarose, digitoxose, and sarmentose), antiarose, digitalose, glucose, and rhamnose; their chemistry is discussed in Chapters 20 and 21. With the exception of glucose and rhamnose, these sugars are not found elsewhere in nature. When either glucose or rhamnose is joined directly to the genin molecule, the union is much firmer than with the other sugars, and the conditions necessary for complete hydrolysis are so drastic that partial dehydration occurs with the production of anhydrogenins, e.g., hydrolysis of ouabain, uzarin, etc. Because the hydrolysis is usually easily effected, Stoll [310] has suggested that the normal sequence of attachment to the C_3—OH is: aglucon—(desoxy sugar)$_x$—(glucose)$_y$.

The Aglucons. With the exception of scillaridin A, the ring system of the aglucons is given in structure I.* In this formulation they are shown with a $\Delta^{\alpha, \beta}$-butenolide C_{17} side chain as indicated by the work of Elderfield [311] and of Ruzicka.[312] Like other steroids, the genins are hydroxylated at C_3, and nearly all are hydroxylated also at C_{14}. The spatial configuration of the ring nucleus, with the exception of uzarigenin, is probably the same as that of coprostane. Scillaridin A appears to be a transitional substance connecting the cardiac glycosides with the toad poisons and is discussed separately. The principal aglucons are listed in Table V.

[310] Stoll and Kreis, *Helv. Chim. Acta*, **16**, 1049, 1390 (1933); Stoll and Renz, *ibid.*, **22**, 1193 (1939).

* The steroidal structure of the cardiac aglucons was suggested first by Kon, *J. Soc. Chem. Ind.*, **53**, 593, 956 (1934), and was based on x-ray measurements made by Bernal and Crowfoot, *ibid.*, **53**, 953 (1934), which showed that they were similar to the sterols and bile acids.

[311] Elderfield et al., *J. Org. Chem.*, **6**, 260, 270, 273, 289 (1941).

[312] Ruzicka, Reichstein, and Fürst, *Helv. Chim. Acta*, **24**, 76 (1941); Ruzicka, Plattner and Fürst, *ibid.*, **24**, 716 (1941).

TABLE V

CARDIAC AGLUCONS *

Aglucon	Position of OH Groups	Variation of Formula I — Rings A/B†	Variation of Formula I — R	Variation of Formula I — Formula	Insoluble Digitonide	M.P. °C.	$[\alpha]_D$ (EtOH)	Sources
Dihydroxygenins								
Adynerigenin ‡	3(α), 14	cis	CH₃	$C_{23}H_{32}O_4$	−	238–241	+18° (pyridine)	Adynerin
Digitoxigenin	3(α), 14	cis	CH₃	$C_{23}H_{34}O_4$	−	250	+19.1	Digitoxin
Thevetigenin	3(β), 14	cis	CH₃	$C_{23}H_{34}O_4$	+	¶		Thevetin
Uzarigenin	3(β), 5 (?)	trans (?)	CH₃	$C_{23}H_{34}O_4$	+	¶		Uzarin
Trihydroxygenins								
Digoxigenin	3(α), 12, 14	cis	CH₃	$C_{23}H_{34}O_5$	−	222	+25.8	Digoxin
Gitoxigenin	3(α), 14, 16	cis	CH₃	$C_{23}H_{34}O_5$		135–137 (235)	+38.5	Gitoxin
Periplogenin	3(α), 5, 14	cis	CH₃	$C_{23}H_{34}O_5$		235	+31.5	Periplocin, periplocy-marin
Sarmentogenin	3(α), 11 (?), 14	cis	CH₃	$C_{23}H_{34}O_5$	−	265–266	+21.5	Sarmentocymarin
Strophanthidin	3(α), 5, 14	cis	CHO	$C_{23}H_{32}O_5$	−	171–175	+44 (MeOH)	k-Strophanthin-β, cymarin
Genins of Uncertain Structure								
Neriantigenin §	3, (?)	(?)	CH₃	$C_{23}H_{32}O_4$		255–259		Neriantin
Convallatoxigenin §	3, 5, 8, 14	(?)	CH₃	$C_{23}H_{32}O_6$		¶		Convallatoxin
Antiarigenin	(?), (?), (?), (?)	(?)	CHO (?)	$C_{23}H_{32}O_7$		¶		α- and β-Antiarin
Ouabagenin	3(α), (?), (?), 14	(?)	CH₂OH(?)	$C_{23}H_{34}O_8$				Ouabain
Scillaridin A ‖	14		CH₃	$C_{24}H_{30}O_3$		245–250	−62.7	Scillaren A

* Data from Sobotka, "The Chemistry of the Steroids," Williams and Wilkins, Baltimore (1938).; from Neumann, Ber., 70, 1547 (1937), and from Trebesohs et al., Ber., 71, 654, 1927 (1938).

† Probable configuration.

‡ Unsaturated at $C_8 : C_9$.

§ Isolated as anhydrogenins.

¶ Unsaturated at $C_9 : C_{11}$ (?).

‖ Lactone ring contains five carbon atoms. See structure XLVI (p. 1446).

The development of the structural chemistry of this group of compounds parallels that of the other steroids.[*] The presence of a lactone side chain as a common feature was noted early in the course of the investigation.[313] The structure of the nucleus to which the lactone ring is attached resisted elucidation for many years, however. The problem was originally attacked by studying the products of oxidative degradation, but again it was selenium dehydrogenation of monoanhydrouzarigenin [314] and of strophanthidin [315] to Diels' hydrocarbon that furnished the essen-
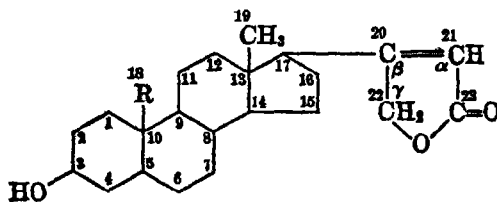
I. Ring system of the cardiac aglucons
(Structure of lactone ring provisional)

tial clue. The precise structural characterization of the ring nucleus and the assignment of the lactone ring to C_{17} have come from the study of the two aglucons, uzarigenin (II) and digitoxigenin (p. 1443). On acid hydrolysis of uzarin, a molecule of water is lost with the sugar moiety and a mixture of the isomeric α_1- and α_2-monoanhydrouzarigenins is formed. After separating the two components of the mixture, Tschesche [314] obtained, by a combination of degradation and reductive procedures, two isomeric lactols, both of which gave etioallocholanic acid when degraded by Barbier-Wieland's method. A similar series of degradations carried out on γ-digitoxanoldiacid (p. 1446) from digitoxigenin by Jacobs and Elderfield [317] led to the production of etiocholanic acid. Both degradations show that the lactone side chain is attached at C_{17}, but neither gives complete information about the spatial configuration of the nucleus. Since most of the cardiac aglucons other than uzarigenin can be correlated with digitoxigenin, it would appear that the normal nuclear configuration is that of etiocholanic acid. This is not necessarily

[*] The earlier work is reviewed by Schmiedeberg, Arch. exptl. Path. Pharmakol., 16, 162 (1883). Subsequent to this, Kiliani, in a series of papers published in the Berichte (1899–1931) and Arch. Pharm. (1895–1913), studied the isolation of the pure glycosides. Modern constitutional investigation began in 1915 with the work of Windaus, reference 320.

[313] Feist, Ber., 31, 534 (1898).

[314] Tschesche and Knick, Z. physiol. Chem., 222, 58 (1933).

[315] Elderfield and Jacobs, Science, 79, 279 (1934); J. Biol. Chem., 107, 143 (1934); cf. Jacobs and Fleck, ibid., 97, 57 (1932).

[316] Tschesche, Z. physiol. Chem., 222, 50 (1933); Angew. Chem., 47, 729 (1934); Z. physiol. Chem., 229, 219 (1934); Ber., 66, 7 (1935); Tschesche and Bohle, Ber., 68, 2252 (1935).

[317] Jacobs and Elderfield, Science, 80, 434, 533 (1934); J. Biol. Chem., 108, 497 (1935); this last paper is very important since it correlates Jacobs' earlier work with the newer concepts.

true, however, for catalytic reduction of a double bond at $C_{14} : C_{15}$ is involved in the degradation of γ-digitoxanol diacid, and in the hydrogenation a spatial configuration different from that of the native aglucons may result.

II. Uzarigenin .

III

IV. 3,21-Diacetoxy- Δ^5-pregnen-20-one

VI. 3-Acetoxy-21-hydroxy-$\Delta^{4,20(22)}$-choladienic acid lactone
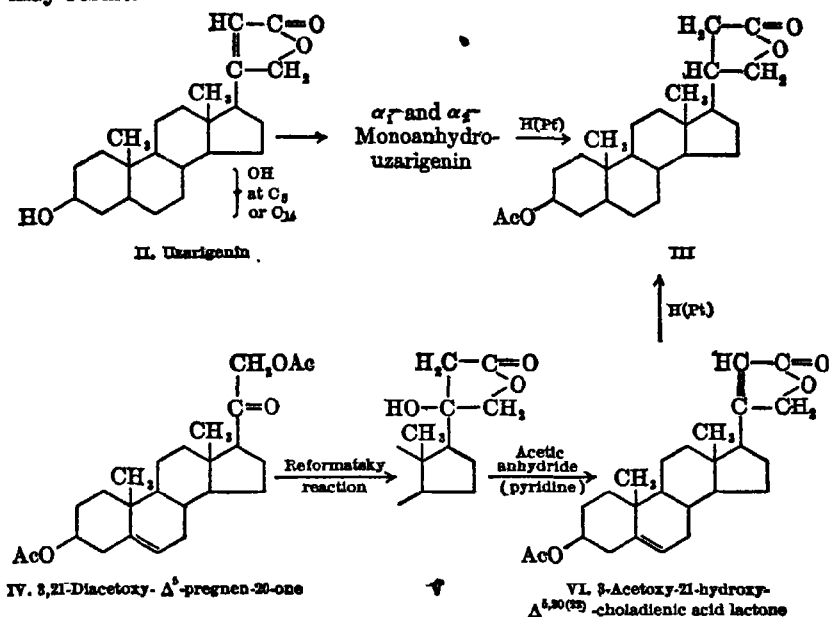
By partial synthesis Ruzicka has obtained additional evidence for the structure of the nucleus and for the position and structure of the lactone ring. Catalytic hydrogenation of the acetates of the monoanhydrouzarigenins gives the corresponding α_1- and α_2-tetrahydromonoanhydrouzarigenin acetates, both of which are stereoisomers of III.[316] These two products appear to be identical with isomers that Ruzicka [312] has obtained by synthesis from 3,21-diacetoxy-Δ^5-pregnen-20-one (IV), the diacetate of an intermediate used in the preparation of desoxycorticosterone (p. 1523). By the Reformatsky reaction, a five-membered hydroxylactone ring (V) was formed at C_{17}. By spontaneous dehydration the hydroxylactone was converted largely to the unsaturated lactone VI. The latter, on catalytic hydrogenation, gave two substances which agree well in their physical properties with α_1- and α_2-tetrahydromonoanhydrouzarigenin acetates, respectively. By similar methods Elderfield [317a] has converted etiocholanic acid via 3,14-bisdesoxythevetigenin to a saturated lactone identical with that obtained from digitoxigenin, sarmentogenin, and digoxigenin by dehydration and hydrogenation; thus a direct correlation between the bile acids and the cardiac aglucons is provided.

[317a] Fried, Linville, and Elderfield, J. Org. Chem., 7, 362 (1942).

The Lactone Ring. These partial syntheses establish the preliminary results of Elderfield,[311] which gave the first indication that the lactone ring is probably unsaturated at $C_{20} : C_{22}$ (IX), rather than at $C_{20} : C_{21}$ (X), as originally formulated by Jacobs. For the purpose of studying the structure of the lactone side chain, Elderfield synthesized a number of β-substituted $\Delta^{\alpha,\beta}$-unsaturated lactones (VII). On comparing the absorption spectra of these and of strophanthidin (XII) or of periplogenin (p. 1443), Elderfield and co-workers noted a similarity in that all absorb strongly at 210–215 mμ. In contrast, vinyl acetate, which has the same arrangement of ethylenic and carbonyl double bonds as is present in a
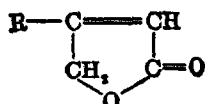
VII. $\Delta^{\alpha,\beta}$-Butenolides
VIIa. R = cyclohexyl
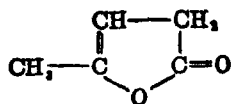
VIII. $\Delta^{\beta,\gamma}$-Angelica lactone
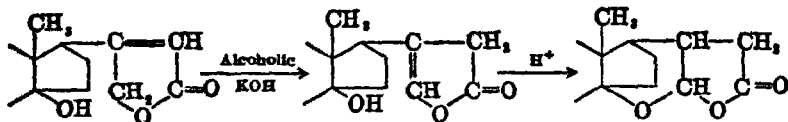
IX. 14-Hydroxyisacon   X. $\Delta^{\beta,\gamma}$-lactone   XI. Isoagiucon

$\Delta^{\beta,\gamma}$-lactone, gives an entirely different absorption curve. This has been confirmed by Ruzicka. In formulating the side chain as a $\Delta^{\beta,\gamma}$-unsaturated lactone, Jacobs[318] was influenced by the characteristic red color reactions (Legal's test) that the cardiac glycosides and the aglucons give when treated with alkaline solutions of sodium nitroprusside. This test is not given by the dihydrogenins in which the lactone ring is saturated. In Jacobs' study, when model experiments with $\Delta^{\alpha,\beta}$- and $\Delta^{\beta,\gamma}$-angelica lactones were conducted with nitroprusside, the latter was found to give the same color reaction as the aglucons; the $\Delta^{\alpha,\beta}$-lactone at first gave a very weak color which grew stronger on standing until finally it was comparable with that of the $\Delta^{\beta,\gamma}$-lactone. Similarly, towards silver solutions there was a parallel in behavior. The $\Delta^{\beta,\gamma}$-lactone reduced ammoniacal silver solutions, the aglucons were weakly reducing, and $\Delta^{\alpha,\beta}$-angelica lactone did not affect the reagent, even on long standing. Using a modified technique for the color tests, Elderfield has found that strophanthidin and β-cyclohexyl-$\Delta^{\alpha,\beta}$-butenolide (VIIa) give identical color reactions, but that $\Delta^{\beta,\gamma}$-angelica lactone shows no similarity. This is particularly true when ferricyanide is employed in place of nitroprusside.

[318] Jacobs, Hoffmann, and Gustus. *J. Biol. Chem.*, **70**, 1 (1926).

According to the $\Delta^{\beta,\gamma}$-lactone formulation of the side chain, an active hydrogen should be present; Jacobs [319] presented evidence in favor of such an active hydrogen, but Elderfield has now shown that this active hydrogen probably resides in the nucleus. The lactone ring of the glycosides and of the aglucons is readily opened by alkali at moderately elevated temperatures, and, on acidification, the reaction is reversed.[320] With alcoholic alkali, however, an irreversible change occurs with most of the genins, and on acidification the so-called isoaglucons are liberated; these are saturated and no longer give the typical color reactions. This change has now been formulated by Elderfield as leading through the stage of the $\Delta^{\beta,\gamma}$-lactone (X) to the isogenins (XI). Participation of a C_{14}—OH group is necessary for the change, and the formation of an isoaglucon is a sensitive test for the presence of such a group. On saponification of the isogenin, an equilibrium mixture of lactol and open forms is produced.[321] Finally Jacobs [318] observed in his early work that the aglucons do not halogenate on titration with bromine. This is not in accord with a $\Delta^{\beta,\gamma}$-lactone formulation, but is compatible with $\Delta^{\alpha,\beta}$-unsaturation.

The Structure of Strophanthidin

The genin strophanthidin has been studied more than any other aglucon, as it is relatively easy to obtain and is not in demand for pharmaceutical preparations. The chemistry of this compound gives a good picture of the type of problem that is met in the study of the aglucons, but the awkward terminology that has grown up for strophanthidin and its derivatives (and those of the other genins) is a real handicap in understanding the various transformations.

Isolation. Strophanthidin (XII) is obtained from the glycosides present in several varieties of *Strophanthus*. From *S. kombé*, for example, a mixture of cymarin and k-strophanthin-β, together with uncharacterized amorphous glycosides, is obtained. Cymarin may be separated from the mixture by dissolving it out with chloroform,[322] and on hydrolysis it yields the aglucon and the sugar cymarose, $C_7H_{14}O_4$.[322] k-Strophanthin-β, in turn, gives strophanthidin and a disaccharide, $C_{13}H_{24}O_9$, composed of cymarose and glucose; glycosidic union with the genin is through cymarose.[323] Aside from the unsaturated lactone ring, strophanthidin possesses a free aldehyde group at C_{10}, tertiary hydroxyl groups situated at C_5 and C_{14}, and a secondary hydroxyl group at C_3.

[319] Jacobs and Elderfield, *ibid.*, **114**, 597 (1936).
[320] Windaus and Hermanns, *Ber.*, **48**, 991 (1915).
[321] Jacobs and Gustus, *J. Biol. Chem.*, **74**, 811 (1927).
[322] Jacobs and Hoffmann, *ibid.*, **67**, 609 (1926).
[323] Jacobs and Hoffmann, *ibid.*, **69**, 153 (1926).

The Lactone Ring. On titration the unsaturated lactone ring of strophanthidin consumes one equivalent of alkali, and on catalytic reduction it absorbs one mole of hydrogen to form a dihydrogenin.[324] That the lactone ring contains four carbon atoms is shown by the oxidation of trianhydrostrophanthidin, which is discussed later.

XII. Strophanthidin   XIII. Isostrophanthidin   XIV. α-Isostrophanthidic acid
                                                              (open form)

XV. Strophanthidinic acid   XVI. α-Isostrophanthidinic acid   XVII. α-Isostrophanthic acid
                                                                    (open form)
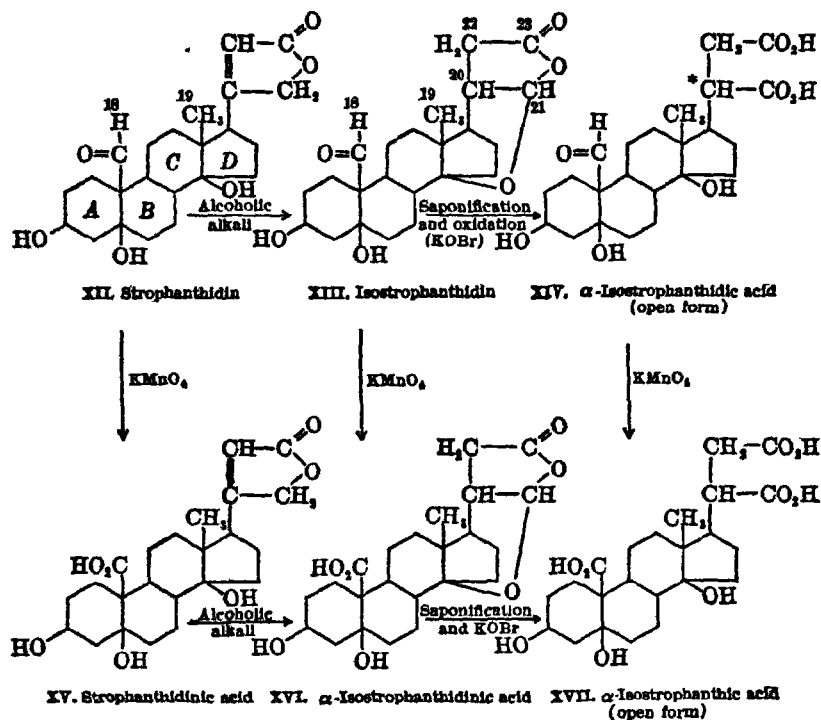
The C_{14}—OH Group. In the change strophanthidin (XII) → isostrophanthidin (XIII) the hydroxyl group involved must be tertiary, since the monoanhydrostrophanthidin produced by the action of alcoholic hydrochloric acid cannot be isomerized. To meet the requirement of being tertiary as well as γ or δ with respect to the aldehyde group of the isomerized side chain, C_{14} is the only position that can be considered for this hydroxyl group.

Some of the products that can be formed from strophanthidin and isostrophanthidin by the proper choice of oxidizing agent are of importance in subsequent arguments and further illustrate the isomerization under consideration. Hypobromite acts selectively on the aldehyde grouping of the lactone ring after saponification, and permanganate will

[324] Jacobs and Heidelberger, *ibid.*, **54**, 253 (1922).

oxidize the C_{10}—CHO group without affecting the side chain if it has not been saponified. By the judicious application of these oxidizing agents all the possible carboxylic acids from strophanthidin and iso-strophanthidin have been realized.[321, 325] The products of the trans-formations are shown in structures XII–XVII. Of these α-isostrophan-thidic (XIV) and α-isostrophanthic (XVII) acids are of greatest impor-tance for subsequent consideration. It should be noted that isostro-phanthidin and all the products derived from it are capable of existing in two forms due to the new center of asymmetry produced at C_{20} as a result of the isomerization.

Further evidence for the attachment of an hydroxyl group at C_{14} comes from the study of dihydrostrophanthidin.[326] By the action of

XVIII. Anhydrodihydrostrophanthidin   XIX

XX   XXI
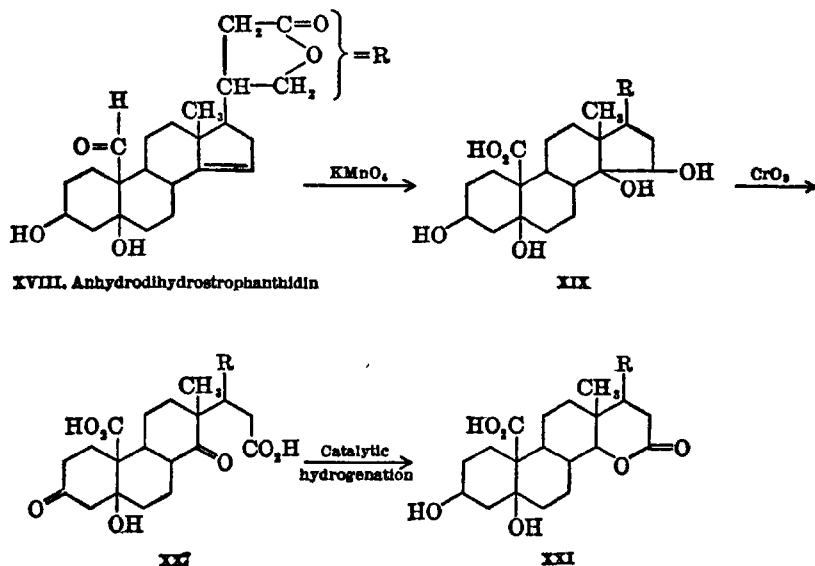
alcoholic hydrogen chloride, the dihydrogenin is converted to an anhy-drogenin (XVIII) which is unsaturated at $C_{14} : C_{15}$. Treatment of XVIII with potassium permanganate produces a glycol (XIX), and, at the same time, the C_{10}—CHO is oxidized to carboxyl. Further oxi-dation with chromic acid opens ring D and converts the C_3—OH to carbonyl (structure XX). On catalytic hydrogenation the carbonyl groups at C_3 and C_{14} are both reduced to hydroxyl groups and the product is isolated as the lactol of structure XXI. The spontaneous for-mation of the lactone indicates a γ- or δ-lactone. The transformation

[321] Jacobs and Collins, ibid., 65, 491 (1925).
[326] Jacobs and Elderfield, ibid., 113, 611 (1936).

XVIII–XXI is explicable only on the basis of an hydroxyl at C_{14} in the parent compound, dihydrostrophanthidin.

The C_{10}—CHO Group. Reduction of the aldehyde group of α-isostrophanthidic acid (XIV) to methyl by the Wolff-Kishner method gives isoperiplogenic acid, which, in turn, can be prepared from digitoxigenin.[227] Since the latter, through its correlation with etiocholanic acid, is known to have methyl groups at C_{10} and C_{13}, the aldehyde group of strophanthidin must be attached at one of these positions, but C_{13} is eliminated by the following considerations: In the production of monoanhydrostrophanthidin by the action of alcoholic hydrochloric acid, the anhydrogenin is isolated as a cyclo-half-acetal. This compound does not possess the properties of an aldehyde or of a secondary alcohol, but on hydrolysis these functions are regenerated. As the secondary hydroxyl group can be shown to be attached at C_3 by other reactions, and as the aldehyde group must be in a γ or δ relationship to this hydroxyl to form a cyclo-acetal, C_{10} is the only possible position of attachment.

The steric relations involved in the formation of the acetal are brought out by a similar lactonization that takes place with the β-isostrophanthidin derivatives.[228] When α-isostrophanthidic acid (XIV)
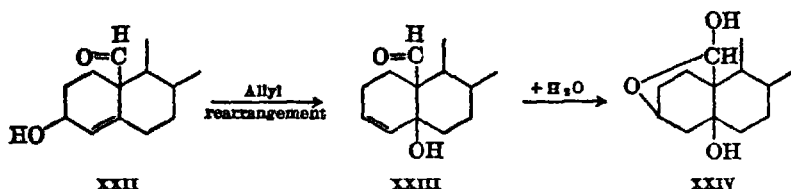
is boiled with alkali, it rearranges to β-isostrophanthidic acid in which the aldehyde group exists both free and as the lactal in combination with the secondary hydroxyl group. On oxidation with permanganate, the aldehyde group at C_{10} is converted to carboxyl, and this acid also readily lactonizes. Since the compounds of the α-isostrophanthidin series do not lactonize in this way, a rearrangement must occur in the alkaline treatment to bring the aldehyde group and the secondary hydroxyl into a cis configuration with respect to each other. Tschesche and Bohle [229] have suggested for the isomerization to the β-series that the treatment with alkali first splits off the C_5—OH; that the resulting unsaturated compound (XXII) then undergoes allyl rearrangement to XXIII; and that water finally adds to the double bond to give an

[227] Jacobs, Elderfield, Grave, and Wignall, ibid., **91**, 617 (1931); Jacobs and Elderfield, ibid., **91**, 625 (1931).

[228] Jacobs and Gustus, ibid., **74**, 829 (1927).

[229] Tschesche and Bohle, Ber., **69**, 2443 (1936).

hydroxyl group at C_3 *cis* to the C_{10}—CHO (structure XXIV). Possibly the same kind of transformation occurs in the formation of the ethyl acetal of monoanhydrostrophanthidin.[330]

By the action of concentrated hydrochloric acid, the C_{10}—CHO group may be brought into reaction with the C_{14}—OH to form the so-called pseudostrophanthidin, to which formula XXV has been assigned.[331] The structure appears to be satisfactory, since pseudostrophanthidin cannot be isomerized, contains a secondary hydroxyl group, and gives the reactions to be described later for the C_5—OH group. The formation of such a compound, however, may involve a steric rearrangement, since spatially strophanthidin appears to resemble *epi*coprosterol and, therefore, the C_{10}—CHO and C_{14}—OH groups are presumably *trans* to each other (*cf.* coprostane model, p. 1368).
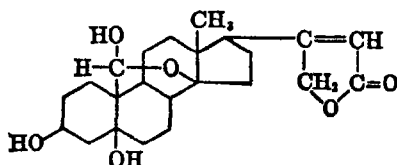
XXV. Pseudostrophanthidin

The C_3—OH Group. The first evidence that an hydroxyl group is attached at C_3 in the cardiac aglucons, and therefore in strophanthidin, was obtained from the study of a derivative of dihydrogitoxigenin (p. 1445) in which all the tertiary hydroxyl groups had been replaced with hydrogen. Vigorous oxidation of this genin derivative cleaved the ring bearing a secondary hydroxyl group and gave a dibasic acid. When the dibasic acid was subjected to thermal decomposition, a pyroketone was obtained. The reaction was carried out by Windaus [332] when the structure of cholesterol was still unknown, and at that time the formation of a pyroketone could not be correctly interpreted. Windaus recognized that the parallel behavior of this diacid and the one formed by similar treatment of cholesterol indicated a correspondence of structure.

The definite placement of a secondary hydroxyl group in strophanthidin at C_3 was later made by a series of reactions analogous to those used in locating the C_3—OH of cholesterol. With α-isostrophanthic dimethyl ester (XXVI) as a starting point, cold chromic acid oxidation gives the corresponding ketone, α-isostrophanthonic dimethyl ester (XXVII). The latter readily loses water to give the unsaturated ketone (XXVIII), which, when treated with ozone, is cleaved in ring A to form

[330] *Cf.* Elderfield, *Chem. Rev.*, **17**, 229 (1935).
[331] Jacobs and Collins, *J. Biol. Chem.*, **63**, 123 (1925).
[332] Windaus, Westphal, and Stein, *Ber.*, **61**, 1847 (1928).

undephanthontriacid dimethyl ester (XXIX).[333] On treatment with weak alkali, this keto acid (XXIX) suffers β-ketone decomposition and is converted to duodephanthondiacid (XXX), and by the action of acetic anhydride-acetyl chloride, the diacid is transformed to an unsaturated lactone (XXXI),[334] which may be catalytically reduced to the saturated dephanthanic acid (XXXII). Barbier-Wieland degradation of dephanthanic acid converts it with the loss of four carbon atoms to dephanthic acid;[335] three of these carbon atoms come from the C_{17} side chain; the fourth is formed by shortening the fragment of ring A. These reactions show that a sequence, $-CH_2-CHOH-CH_2-$, is present in one of the rings and terminates at a tertiary carbon. This sequence can be accommodated only in ring A, and the requirement of termination in a tertiary carbon atom places the hydroxyl group definitely at C_3.

Since strophanthidin does not give an insoluble digitonide, the C_3—OH group is probably in an α-configuration with respect to the C_{10}—CHO.[336] The evidence is inconclusive, however, since cholestanetriol (3,5,6-trihydroxy), in which the C_3—OH is presumably β to the C_{10}—CH₃, fails to give an insoluble digitonide.

The C_5—OH Group. As mentioned above, the dehydration of α-isostrophanthonic acid (XXVII) proceeds with great ease. Because such dehydrations are typical of β-hydroxyketones, a tertiary hydroxyl group is placed at C_5, for only at this position can an hydroxyl group be both β to C_3 and tertiary. The C_5—OH group is probably *cis* to the C_{10}—CHO. This is shown by the following evidence from the work of Jacobs and Elderfield:[337] When dihydrostrophanthidin is treated with hydrogen cyanide, the C_{10}—CHO is converted *via* a cyanohydrin to two isomeric α-hydroxy acids. Both these acids readily form lactones (so-called homolactones) through interaction with the C_5—OH. The involvement of this hydroxyl group in the formation of homolactones is shown by the fact that anhydrostrophanthidin gives similar reactions, and that the lactones can be converted to ketones (OH at C_3) or formed after protection of the C_3—OH by benzoylation. Tschesche[329] has pointed out that this ease of lactone formation indicates a *cis* relationship between the C_5—OH and the C_{10}—CHO. (*Cf.* Alder-Stein rule, p. 1376.)

The Anhydrostrophanthidins. Dehydration of monoanhydrostrophanthidin to dianhydrostrophanthidin is effected by heating the acetal of anhydrostrophanthidin with alcoholic hydrogen chloride. The

[333] Jacobs and Gustus, *J. Biol. Chem.*, **79**, 539 (1928).

[334] Jacobs and Gustus, *ibid.*, **92**, 323 (1931).

[335] Jacobs and Elderfield, *ibid.*, **102**, 237 (1933).

[336] Tschesche and Bohle, *Ber.*, **68**, 2252 (1935).

[337] Jacobs and Elderfield, *J. Biol. Chem.*, **113**, 625 (1936).

$$CH_2-CO_2CH_3$$

$$CH-C=O$$

XXVI. α-Isostrophanthic acid dimethyl ester

Cold
CrO₃

XXVII. α-Isostrophanthonic acid dimethyl ester

Dehydration

XXVIII. Anhydroisostrophanthonic acid dimethyl ester

O₃

XXIX. Undephanthontriacid dimethyl ester

Weak alkali

$$CH_2-CO_2H$$

$$CH-C=O$$

XXX. Duodephanthondiacid

Acetic anhydride-
Acetyl chloride

XXXI. Unsaturated lactone

Catalytic reduction

$$CH_2-CO_2H$$

$$CH-CO_2H$$

XXXII. Dephanthanic acid

Barbier-Wieland
degradation

$$CO_2H$$

XXXIII. Dephanthic acid

product obtained is the ethyl hemiacetal of dianhydrostrophanthidin (XXXIV), formed by the loss of the C_5—OH. When dianhydrostrophanthidin is treated with concentrated aqueous hydrochloric acid, another molecule of water is lost with the formation of trianhydrostrophanthidin (XXXV).[331] With the exception of the double bond of the lactone ring, the trianhydrogenin shows none of the properties of an unsaturated compound and, when oxidized with fuming nitric acid, yields 1,2,3,4-benzenetetracarboxylic acid.[338] The production of this tetracarboxylic acid is explicable if, in the formation of trianhydrostrophanthidin, the C_{10}—CHO wanders to C_1 with a simultaneous shift of bonds to produce aromatization of ring B. Fieser [339] has suggested that the reaction may be explained by an enlargement of ring A to a seven-membered ring, rather than a shift of the aldehyde group. There is some question as to whether this change takes place in the production of trianhydrostrophanthidin or whether it has already occurred in the formation of the dianhydrogenin.[340] The lactone ring of strophanthidin is, in general, quite resistant to oxidizing agents. In trianhydrostrophanthidin, however, the ring is easily oxidized away to give the acid of the probable structure XXXVI.[341]
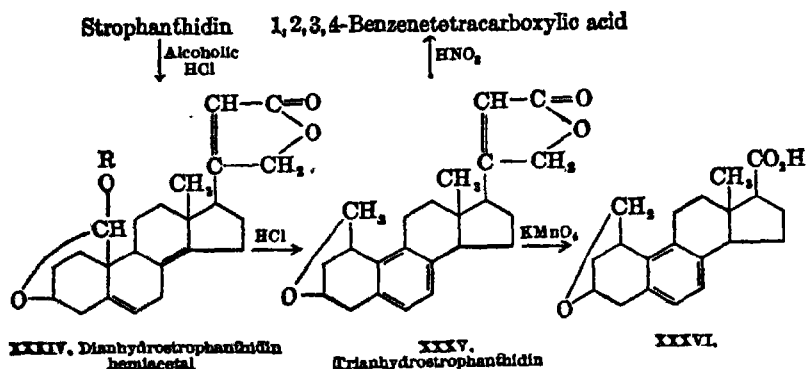
In the previous discussion the nuclear double bond in monoanhydrostrophanthidin has been assigned to $C_{14} : C_{15}$. This is apparently true for alkaline media, but in certain reactions in acid or neutral media there is evidence that the double bond shifts to $C_8 : C_{14}$ (*cf.* α-stenols and apocholic acid). In dianhydrostrophanthidin there is another anomaly. When the lactone side chain of monoanhydrostrophanthidin is saponi-

[338] Tschesche and Knick, Z. physiol. Chem., **229**, 233 (1934).
[339] Fieser, "The Chemistry of Natural Products Related to Phenanthrene," Reinhold Publishing Corp., New York (1937), p. 274.
[340] Elderfield, Chem. Rev., **17**, 225 (1935).
[341] Jacobs and Gustus, J. Biol. Chem., **74**, 805 (1927).

fied, the aldehydo acid obtained does not relactonize.[342] Apparently this is due to a rearrangement of the lactone double bond into ring D to form a conjugated system $C_{14} : C_{15} \cdot C_{16} : C_{17}$. The double bonds of monoanhydro- and dianhydro-strophanthidin can be preferentially hydrogenated; as in certain of the sterols, the nuclear double bonds are hydrogenated more easily than the double bond of the side chain.[331]

The stereochemistry of these anhydrostrophanthidins and certain other unexplained products such as the γ- and δ-strophanthidin series [328] is a problem of the future.

Interrelationship of the Aglucons

Through conversion to mutually common compounds the aglucons have been correlated with each other, and, by various special methods, the individual characteristics of the genins have been ascertained. Brief summaries of the work leading to the currently accepted structures follow.

Periplogenin. As cited earlier (p. 1438), isoperiplogenic acid may be formed by reduction of the C_{10}—CHO of α-isostrophanthidic acid (XIV) to C_{10}—CH_3. Periplogenin is, therefore, a desoxostrophanthidin.

Digitoxigenin.[343] Oxidation with chromic acid of the methyl ester of isoperiplogenic acid converts it to a ketonic ester (carbonyl at C_3), which readily loses water (C_5—OH) to form an anhydro ketone. On hydrogenation of the anhydro compound, a mixture of stereoisomeric dihydro compounds is obtained; one of these (isodigitoxigonic ester) is identical with an ester formed from digitoxigenin. The two hydroxyl groups of digitoxigenin must be attached at C_3 and C_{14}. Digitoxigenin does not form an insoluble digitonide, and the C_3—OH must be in an α-configuration. The degradation of digitoxigenin to etiocholanic acid *via* γ-digitoxanol diacid (p. 1432) shows that the relation of rings A/B is *cis*.

Adynerigenin.[344] The aglucon adynerigenin is hydroxylated at C_3 and C_{14} and appears to be unsaturated in the ring system at $C_8 : C_9$. This nuclear double bond is resistant to hydrogenation, but, like isodehydrocholesterol (p. 1386), both the genin and the monoanhydrogenin can be hydrogenated in the presence of hydrochloric acid. The hydrogenation product is identical with the saturated lactone from digitogenin. The C_3—OH group of adynerigenin has the α-configuration, since the genin does not form an insoluble digitonide.

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[342] Jacobs and Elderfield, *ibid.*, **108**, 693 (1935).

[343] Jacobs and Elderfield, *ibid.*, **92**, 313 (1931).

[344] Neumann, *Ber.*, **70**, 1547 (1937); Tschesche and Bohle, *Ber.*, **71**, 654 (1938) Tschesche, Bohle, and Neumann, *Ber.*, **71**, 1927 (1938).

Thevetigenin.[345] When thevetin is hydrolyzed with hydrochloric acid, two molecules of glucose and one of water are split off to give monoanhydroprothevetigenin, a partially desugared product which probably contains digitalose. The hydroxyl group lost in the hydrolysis is presumably attached at C_{14}, since potassium hydroxide isomerizes the glycoside but not the hydrolytic product. After the partially desugared genin is saturated to a tetrahydro compound, it may be completely hydrolyzed. The resulting tetrahydroanhydrothevetigenin is converted by careful oxidation with chromic acid into tetrahydroanhydrodigitoxigenone, thus placing the second hydroxyl group at C_3. The C_3—OH group must have a β-configuration, since anhydrothevetigenin forms an insoluble digitonide.

Uzarigenin. The aglucon uzarigenin (II) has been isolated only as $α_1$- and $α_2$-monoanhydrouzarigenins. The position of the hydroxyl group lost in the dehydration is uncertain. Tschesche [317] at first placed this tertiary hydroxyl group at C_5 and later transferred it to C_{14}, because treatment of uzarin with alkali produced a change which seemed to be analogous to the isomerization of the other aglucons. Ruzicka [312] has questioned this analogy and favors attachment at C_5 of the tertiary hydroxyl group lost in the dehydration. In line with this, the physiological properties of uzarin are quite different from those of the other glycosides. A second hydroxyl group has been placed at C_3, since the monoanhydrouzarigenins form insoluble digitonides.

Digoxigenin.[346] Although the aglucon digoxigenin contains two secondary and one tertiary hydroxyl (C_{14}) groups, the diketone resulting from oxidation of the genin gives mono derivatives with ketone reagents. The inertness of this one carbonyl group at first was difficult to explain and led, in conjunction with other reactions, to the assumption of an hydroxyl group at C_{11}. Mason and Hoehn,[346] however, have degraded digoxigenin and desoxycholic acid to identical diketocholanic acids, and therefore the two hydroxyl groups which give rise to keto groups are placed at C_3 and at C_{12}. The hydroxyl group at C_3 has an α-configuration, while that at C_{12} may be epimeric to the C_{12}—OH group of desoxycholic acid.

Gitoxigenin. The aglucon gitoxigenin (XLI) possesses one tertiary (C_{14}) and two secondary hydroxyl groups. One of these secondary groups is attached at C_3, and the other has been placed at C_{16}. This second hydroxyl group enters into reactions with the unsaturated lactone side chain, so that alkaline isomerization of this genin is somewhat

[345] Elderfield, *J. Biol. Chem.*, **115**, 247 (1936); Tschesche, *Ber.*, **69**, 2368 (1936).

[346] Smith, *J. Chem. Soc.*, **508** (1930); 23 (1931); 1050, 1305 (1935); 354 (1936); Tschesche and Bohle, *Ber.*, **69**, 793 (1936); Mason and Hoehn, *J. Am. Chem. Soc.*, **60**, 2024 (1938).

different from the normal.[247] The isogenin (XXXVII), formed by treat-
ment with alkali, is unusually stable, and the lactone of the oxidation
product, isogitoxigenic acid, is relatively resistant to hydrolysis. If
gitoxigenin is oxidized with chromic acid to diketogitoxigenin, the prod-
uct does not give a positive Legal reaction. This is probably due to
a spontaneous formation of an isogenone through interaction with the
C_{14}—OH. The mechanism of the reaction may be a shift of the double
bond of the lactone side chain into conjugation with the carbonyl double
bond at C_{16}. The probable structure of the isogenone is shown in for-
mula XXXVIII.

On hydrogenation, gitoxigenin is converted into two isomeric α- and
β-dihydrogenins.[248] Both these dihydrogitoxigenins undergo mutaro-
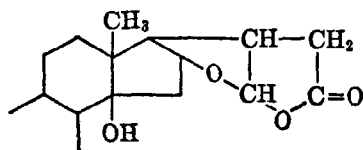
XXXVII.
Isogitoxigenin
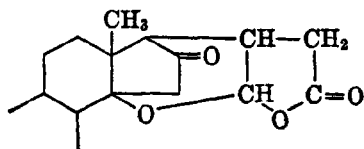
XXXVIII.
Isogitoxigenone
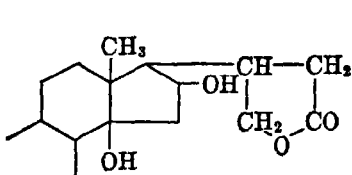
XXXIX.
α-Dihydrogitoxigenin (dextro)
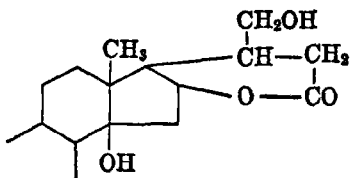
XL.
β-Dihydrogitoxigenin (levo)

tation, probably through a rearrangement involving the lactone group.
Structures XXXIX and XL represent the probable configurations of
these two dihydrogitoxigenins. As is evident from these structures, in
the β-form lactonization has occurred on the secondary hydroxyl group
at C_{16}. On oxidizing the α-dihydrogenin, a dihydrogitoxigenone is
obtained. This ketone is easily dehydrated, as would be expected of a
compound in which the tertiary hydroxyl group is in a β-position with
respect to the carbonyl group.

Gitoxigenin (XLI) has been correlated with digitoxigenin by the
following reactions:[249] After isomerization the isogitoxigenin was sapon-

[247] Jacobs and Gustus, J. Biol. Chem., 79, 553 (1928); 82, 403 (1929); 88, 531 (1930).

[248] Jacobs and Elderfield, ibid., 100, 671 (1933). Cf. Windaus et al., reference 332.

[249] Jacobs and Gustus, J. Biol. Chem., 86, 199 (1930). Cf. Windaus and Freese, Ber.
58, 2503 (1925), and earlier papers.

ified and oxidized to isogitoxigenic acid (XLII), which differs from the usual isogenic acids in that lactonization takes place with the C_{16}—OH. The C_{14}—OH was converted to a chloride, and the chloro acid transformed to an unsaturated acid (XLIII) by splitting out hydrogen chloride. On catalytic hydrogenation the lactone ring was opened, the C_{16}—OH group replaced, and the double bond saturated. The resulting acid was the digitoxanoldiacid (XLIV) previously mentioned (p. 1432).

Oleandrin is an acetyl glycoside of gitoxigenin.[350] Hydrolysis of oleandrin with acid gives the sugar oleandrose, $C_7H_{14}O_4$ (a methyl ether of a methyldesoxypentose), and the genin oleandrigenin, $C_{25}H_{36}O_6$. Alkaline hydrolysis converts oleandrigenin to gitoxigenin and acetic acid. In oleandrin and oleandrigenin, gitoxigenin is acetylated at C_{16}.

XLI. Gitoxigenin   XLII. Isogitogenic acid

−HOH
(via chloride)

XLIII   XLIV. γ-Digitoxanoldiacid
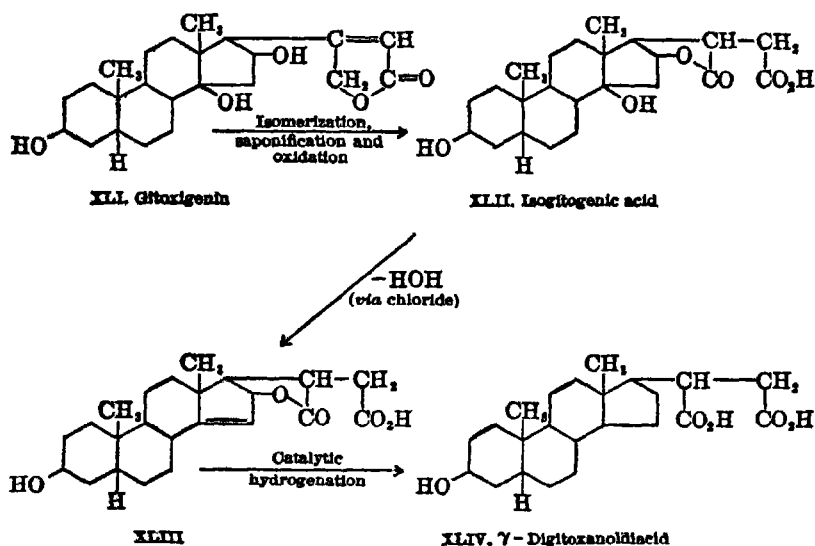
Sarmentogenin.[351] There are three nuclear hydroxyl groups in sarmentogenin, two of which have been placed at C_3 and C_{14}. The third hydroxyl group has been assigned either to C_{11} or to C_{12} by the following considerations: Hydrolysis of the glycoside sarmentin with alcoholic hydrochloric acid eliminates the C_{14}—OH to produce α-tetrahydroanhydrosarmentogenin. From the latter, a diketone is obtained on oxidation, and one of the two carbonyl groups of this diketone is unreactive toward ketone reagents. This is typical of a carbonyl group

[350] Neumann, *Ber.*, **70**, 1547 (1937); Tschesche, *Ber.*, **70**, 1554 (1937).

[351] Tschesche and Bohle, *Ber.*, **69**, 2497 (1936); Mason and Hoehn, *J. Am. Chem. Soc.*, **60**, 2024 (1939); cf. Jacobs and Hoffmann, *J. Biol. Chem.*, **79**, 531 (1928); Tschesche, *Ber.*, **66**, 423 (1935).

at C_{11} or C_{12}, as has been shown from the study of digoxigenin, of certain of the dehydrocholic acids,[352] and of the adrenal substances (p. 1510).*

Genins of Uncertain Structure. The glycoside neriantin is obtained in traces from the residues of the commercial manufacture of oleandrin. Because of the small amount available for study, only a cursory examination of **neriantigenin** [353] has been possible. The genin appears to have the typical C_3—OH group, a secondary hydroxyl group elsewhere in the nucleus, and a nuclear double bond that can be catalytically reduced. **Convallatoxigenin,**[354] from the ·glycoside found in the lily-of-the-valley, is likewise unsaturated in the nucleus, presumably at $C_9 : C_{11}$. In addition to hydroxyl groups at C_3 and C_{14}, it appears to have two tertiary hydroxyl groups at C_5 and C_8, respectively. The two glycosides, β-antiarin and ouabain, have the sugars so firmly attached that hydrolysis gives anhydrogenins in each case. **Antiarigenin** [355] may be an hydroxylated strophanthidin. **Ouabagenin** [356] appears to have two unplaced hydroxyl groups in addition to those assumed to be at C_3 and C_{14}. On acetolysis, anhydroouabagenin loses formaldehyde with the formation of a benzenoid ring in the nucleus. To explain this a C_{10}—CH$_2$OH group has been postulated.

In addition to these genins that are derivatives of the type formula I, there is an aglucon, **digigenin,**[357] $C_{21}H_{28}O_4$, which does not fit this formulation. Digigenin and the sugar diginose are obtained by hydrolyzing diginin, a glycoside from *digitalis purpurea*. As the glycoside is without physiological activity, and as the genin does not contain the lactone ring, it is questionable whether they should be regarded as members of this group.

[352] Longwell and Wintersteiner, *J. Am. Chem. Soc.*, **62**, 200 (1940); *cf.* Marker and Lawson, *ibid.*, **60**, 1334 (1938).

* In 1936, from a study of the corresponding products from digoxigenin and from sarmentogenin, Tschesche (see reference 351) concluded that both were hydroxylated at C_{11}, and that they differed in the steric configuration of rings B/C. To digoxigenin was assigned the normal *trans* configuration, and to sarmentogenin the abnormal *cis* configuration of these rings. Such an argument is no longer necessary to explain the facts, although future work may show it to be valid.

[353] Tschesche, Bohle, and Neumann, *Ber.*, **71**, 1927 (1938).

[354] Jacobs and Bigelow, *J. Biol. Chem.*, **96**, 647 (1932); **101**, 15 (1933); Fieser and Newman, *ibid.*, **114**, 705 (1936); Tschesche, *Ber.*, **70**, 43 (1937).

[355] Kiliani, *Ber.*, **43**, 3574 (1910); **46**, 667, 2179 (1913); Tschesche and Haupt, *Ber.*, **69**, 1377 (1936).

[356] Karrer, *Helv. Chim. Acta*, **12**, 506 (1929); Tschesche and Haupt, *Ber.*, **69**, 459 (1936); Fieser and Newman, *J. Biol. Chem.*, **114**, 705 (1936); Tschesche and Haupt, *Ber.*, **70**, 43 (1937); Chakravorty and Wallis, *J. Am. Chem. Soc.*, **60**, 1379 (1938); Marker and Shabica, *ibid.*, **64**, 720 (1942).

[357] Karrer, *Festschrift Emil C. Barrell*, Basel (1936), p. 238 [*C. A.*, **31**, 2347 (1937)]; Shoppee and Reichstein, *Helv. Chim. Acta*, **23**, 975 (1940).

The Squill Aglucon

The glycosides scillaren A (XLV) and B have been isolated from the bulbs of the squill (*Scilla maritima*) by Stoll,[155] but only the former has been obtained in pure form. Scillaren A on catalytic hydrogenation is reduced to a hexahydrodesoxy acid which on treatment with methanolic hydrogen chloride is desugared and dehydrated to a mono-unsaturated product. Catalytic hydrogenation of the latter in a neutral medium gives 3(β)-hydroxy*allo*cholanic acid. The formation of this acid shows clearly that the lactone ring of scillaren A is six-membered and that a 3(β)-hydroxyl group is present. Assignment of unsaturation
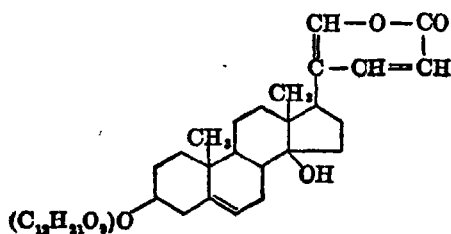
XLV. Scillaren A
(provisional)
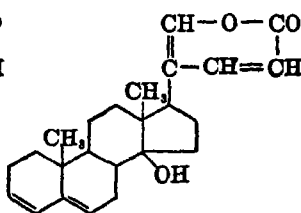
XLVI. Scillaridin A
(provisional)

to C_5 is provisional, however. Although catalytic hydrogenation of the genin leads to an *allo* structure, enzymatic followed by acid hydrolysis gives first proscillaridin A and glucose, and then, with loss of rhamnose and water, scillaridin A (XLVI). The formulation of the latter is provisional since its absorption spectrum shows a maximum at 290–300 mμ rather than three maxima corresponding to those of $\Delta^{3,5}$-cholestadiene
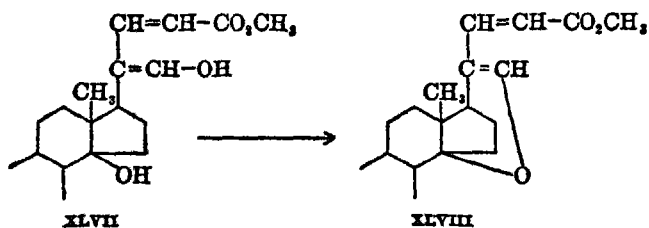
XLVII

XLVIII

(p. 1395). The tertiary hydroxyl group of scillaren A is assigned to C_{14} as in the other aglucons. When scillaridin A is treated with methanolic potassium hydroxide the lactone ring is opened with the formation of an ester, provisionally formulated as XLVII, rather than a potassium salt. This ester readily loses water to form a derivative (XLVIII) of isoscillaridin A. Scillaren A and its derivatives do not give Legal's test.

[155] Stoll *et al., Z. physiol. Chem.,* **222**, 24 (1933); *Helv. Chim. Acta*, **17**, 641, 1334 (1934)· **18**, 82, 120, 401, 644, 1247 (1935); **24**, 1380 (1941).

The Toad Poisons

Venom secreted in the parotid gland of toads is a complicated mixture consisting of several conjugated and free genins. Other substances present include epinephrine,[359] bufotenidine and related tryptamines,[360] sterols,[361] fats, etc.* The toad poisons proper are suberylarginine derivatives of the genins. The nature of the genins, or bufagins, as they are called, varies with the species, and they may be differentiated by means of a prefix indicating the toad from which they are isolated.[362] As a result of enzyme action, hydrolysis of the venom to the free genins occurs in the toad secretion, but with acid hydrolysis dehydration takes place and only anhydrogenins are obtained. The bufagins are polyhydroxy or acetylated hydroxy lactones, containing 23 or 24 carbon atoms, and are more closely allied with scillaridin A than with the other aglucons. Many of them give a positive Liebermann test, and none gives Legal's test.

Marinobufagin (or bufagin), $C_{24}H_{34}O_5$, m.p. 213°, from the toad *Bufo marinus*, was the first genin to be isolated,[363] but bufotalin, $C_{24}H_{34}O_5(CH_3CO)$, m.p. 148°, from the common European toad, *Bufo vulgaris*, has been studied more extensively.[364] From Ch'an Su, or senso, the dried secretion of the Chinese toad (*Bufo gargarizans*), cinobufagin, $C_{24}H_{32}O_5(CH_3CO)$, m.p. 223°, together with other bufagins, has been obtained.[365]

The toad poisons are known to be steroids through the isolation of Diels' hydrocarbon from the products of selenium dehydrogenation of pseudobufotalin,[366] of cinobufagin,[365] and of marinobufagin.[366] On the other hand, only impure chrysene has been obtained by selenium dehy-

[359] Jensen and Chen, *J. Biol. Chem.*, **82**, 397 (1929); **87**, 741 (1930); Deulofeu, *Z. physiol. Chem.*, **237**, 171 (1935).

[360] Wieland, Konz, and Mittasch, *Ann.*, **513**, 1 (1934).

[361] Chen, Jensen, and Chen, *Proc. Soc. Exptl. Biol. Med.*, **29**, 905 (1932); Hüttel and Behringer, *Z. physiol. Chem.*, **245**, 175 (1937).

* Cholesterol and γ-sitosterol are the sterols that have been identified in toad poisons, but ergosterol may be present also. It is remarkable that a plant sterol should be found in a secretion of a member of the animal kingdom.

[362] Chen and Chen, *J. Pharmacol.*, **49**, 561 (1933).

[363] Abel and Macht, *ibid.*, **3**, 319 (1911); Jensen and Evans, Jr., *J. Biol. Chem.*, **104**, 307 (1934); Jensen, *J. Am. Chem. Soc.*, **59**, 767 (1937).

[364] Wieland, Hesse, and Hüttel, *Ann.*, **524**, 203 (1936). In this reference a good method of isolating the toad poisons is given.

[365] Kondo and Ikawa, *J. Pharm. Soc. Japan*, **53**, 2 (1933) [*Chem. Zentr.*, (II) 2558 (1933)]; Tschesche and Offe, *Ber.*, **68**, 1998 (1935); Jensen, *J. Am. Chem. Soc.*, **57**, 2733 (1935); Tschesche and Offe, *Ber.*, **69**, 2361 (1936); Kotake and Kuwada, *Sci. Papers Phys. Chem. Research*, (*Tokyo*), **36**, 106 (1939) [*C. A.*, **33**, 7304 (1939)]; Kondo and Ohno, *J. Pharm. Soc. Japan*, **59**, 186 (1939) [*Chem. Zentr.*, (I) 1997 (1940)].

[366] Ikawa, *J. Pharm. Soc. Japan*, **55**, 748 (1935) [*C. A.*, **29**, 7341 (1935)].

drogenation of bufotalin.[367] The formation of this hydrocarbon does not invalidate the argument, since it is also produced from other members of the group. These degradations are supported by x-ray measurements on bufagin, which show that the molecule is comparable to that of the sterols.[368]

Most of the structural details of the bufagins have been suggested by Wieland[364] and by Tschesche.[365] The genins and a number of the transformation products absorb light at 290–300 mμ and react with methanolic potassium hydroxide to form esters in the same way as scillaridin A. The two investigators have independently concluded that the lactone side chain is the same in the bufagins as in scillaridin A. An especially convincing argument is the similarity of the absorption curves of the bufagins and of the simple molecule, cumalinic acid (XLIX).[365] In accord with this formulation of the lactone side chain, ozonization of the bufagins gives glyoxylic and formic acids.[364]

$$HO_2C—C—CH=CH$$
$$HC—O—C=O$$

XLIX. Cumalinic acid
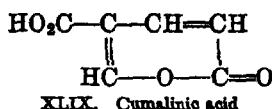
Bufotalin. Wieland[364] has suggested structure L (or L*a*) for bufotalin. This bufagin contains a secondary hydroxyl, a tertiary hydroxyl, and a tertiary acetoxy group. The tertiary hydroxyl and acetoxy groups are easily split off by cold hydrochloric acid to give the quadruply unsaturated bufotalien (LI or LI*a*). Since bufotalone, the ketone from bufotalin, is isomerized when treated at 0° with dilute alkali, the tertiary hydroxyl group removed in the acid treatment is probably attached at C_{14}. The tertiary acetoxy group must be attached at C_9 or C_5, because the absorption spectrum of bufotalien (max. 290–300 mμ) does not indicate a conjugated system in the ring, and attachment at C_8 would lead to such a system. The secondary hydroxyl group is placed at C_3, as in the sterols.

Bufotalien is obtained when bufotoxin is hydrolyzed with hydrochloric acid. Wieland has suggested that the suberylarginine group is attached at the C_{14}—OH in the toxin, but the mode of linkage to the nitrogenous moiety is uncertain. On catalytic reduction of acetylbufotalien two products result: acetylbufotalan and a by-product, an acetylcholanic acid formed by reduction and fission of the lactone side chain. When this acetylcholanic acid is converted to a cholanic acid,

[367] Wieland and Hesse, Ann., 517, 22 (1935).
[368] Crowfoot, J. Soc. Chem. Ind., 54, 568 (1935).

isobufocholanic acid, m.p. 179°, $[\alpha]_D + 50.5°$, is obtained.[369] It is not identical with any of the known cholanic acids (Table II).
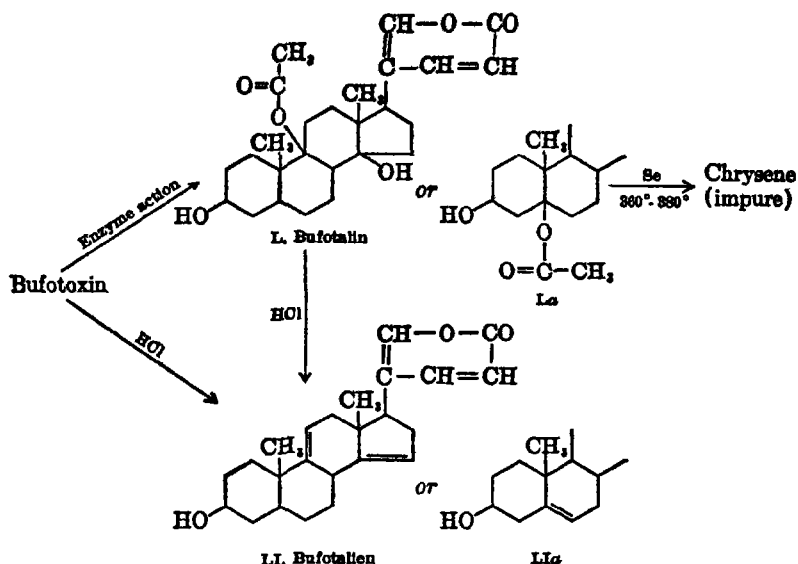
Cinobufagin. The work of Tschesche [365] indicated the presence of hydroxyl groups at C_3 and C_{14}, and of a six-membered lactone ring in cinobufagin (LII). An acetoxy group and a nuclear double bond were unplaced. Kuwada [370] has assigned the acetoxy group to C_{12} and the

LII. Cinobufagin
(provisional)
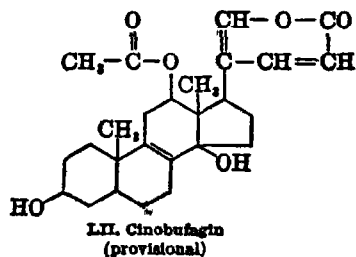
nuclear double bond to $C_8 : C_9$. A series of transformations designed to establish these details was unsuccessful, however, and formula LII must be regarded as provisional.

[369] Wieland, Hesse, and Meyer, *Ann.*, **493**, 272 (1932). For earlier work see Wieland and Weil, *Ber.*, **46**, 3315 (1913); Wieland and Alles, *Ber.*, **35**, 1789 (1922).

[370] Kuwada and Kotake, *Sci. Papers Phys. Chem. Research (Tokyo)*, **35**, 419 (1939) [*C. A.*, **33**, 5861 (1939)]; Kuwada, *J. Chem. Soc. Japan*, **60**, 45 (1939) [*C. A.*, **34**, 1031 (1940)].

. **Other Toad Poisons.** In all, some twelve bufagins have been isolated. The most important are gamabufagin, $C_{24}H_{34}O_5$, m.p. 213°, from the dried skins of the Japanese toad;[371] arenobufagin, $C_{23}H_{31}O_5$-(CH_3CO), m.p. 220°, from the Argentine toad;[372] and regularobufagin, $C_{23}H_{31}O_5(CH_3CO)$, m.p. 236°, from the South African toad.[372] The physical constants of these bufagins and of the poisons have been tabulated by Dane[373] and by Schoppee.[374]

Structure and Physiological Action *

The effect on the diseased heart and the emetic action are the important physiological properties of the cardiac principles. Their structure and activity can be correlated in a general way. The potency of the cardiac principles is dependent on the presence, and on the steric position, of the unsaturated lactone ring; on the presence of the sugar or nitrogenous moiety; on the presence of an hydroxyl group at C_{14}; and on the absence of nuclear unsaturation at $C_8 : C_9$. The potency is modified by the spatial configuration of rings A/B, and by the nature of the sugar moiety. It is little affected by the steric position of the C_3—OH group.

The Heart Action. The cardiotonic properties of the drugs are due to a direct action on the heart muscle. The response obtained with healthy tissue differs somewhat from that with diseased, so that animal experimentation is not directly comparable with clinical experience. Experimentally, the physiological potency is determined by injecting an aqueous-alcoholic solution of the glycoside into the blood stream of cats (Hatcher-Brody method), or an aqueous solution into the lymph sac of frogs. The smallest amount of substance necessary to produce systolic standstill of the heart is determined, and the so-called minimum lethal dose (M.L.D.) calculated. In Table VI physiological data on a number of the pure glycosides and on bufotalin are given.

From the data of Table VI it is evident that the carbon content of the unsaturated lactone ring does not markedly affect the potency. Reduction of the lactone ring, in the cardiac glycosides that have been examined, reduces the potency over a hundredfold,[375] and isomerization by enzymes or by alkali, likewise destroys the potency.[376] The C_{14}—OH

[371] Wieland and Vocke, *Ann.*, **481**, 215 (1930); Kondo and Ohno, reference 365.

[372] Jensen, *J. Am. Chem. Soc.*, **57**, 1765 (1935). The earlier literature is cited in this publication.

[373] Dane, *Tabulae Biologicae Periodicae*, **8**, 204 (1933).

[374] Schoppee, *Ann. Rev. Biochem.*, **11**, 103 (1942).

* Books on pharmacology: Cushny, "Digitalis and Its Allies," Longmans, Green & Co., London (1925); Weese, "Digitalis," Thieme, Leipzig (1936).

[375] Jacobs, *Physiol. Rev.*, **18**, 222 (1933).

[376] Jacobs, *J. Biol. Chem.*, **88**, 519 (1930); Lamb and Smith, *J. Chem. Soc.*, 422 (1936).

TABLE VI

PHYSIOLOGICAL POTENCY OF THE CARDIAC PRINCIPLES [*]

(Expressed in milligrams per kilogram of cat, or milligram per gram of frog)

Drug	Cat Units	Frog Minimal Systolic Dose	Minimal Emetic Dose in Cats
Four-Carbon Lactone Ring			
Convallatoxin	0.08	0.00021	0.060
β-Antiarin	0.10	0.00039	0.040
Ouabain	0.12	0.00050	0.060
α-Antiarin	0.13	0.00050	0.06
Cymarin	0.13	0.00060	0.080
Periplocymarin	0.15	0.00318	0.080
Oleandrin	0.20	0.00127	0.040
Digoxin	0.22	0.00250	0.075
Digitoxin	0.33	0.00800	0.150
Thevetin	0.92	0.00450	0.225
Uzarin	5.08	1.50000	0.350
Five-Carbon Lactone Ring			
Bufotalin	0.13	0.00917	0.05
Scillaren A	0.15	0.00070	0.100

[*] Data from Chen and co-workers, *J Am. Pharm. Assoc.*, **25**, 579 (1936); **26**, 214 (1937); **27**, 113 (1938).

is assumed to play an important role in the production of heart action, since neriantin is devoid of cardiotonic properties, and its genin is thought to lack an hydroxyl group at C_{14}.[344] Similarly adynerin is without cardiotonic properties, and, as adynerigenin differs from the active dihydric aglúcones by the presence of a double bond at $C_8 : C_9$, it is reasoned that such nuclear unsaturation destroys potency.[344] From the values for the three glycosides, digitoxin, thevitin, and uzarin, it is probable that a *cis* configuration of rings A/B is essential for high potency. The genin of uzarin apparently has a *trans* configuration of rings A/B, and its potency is far lower than that of digoxin and of thevetin in which rings A/B are *cis*. The small effect of epimerization of the C_3—OH is brought out by comparison of digitoxin with thevetin. Digitoxin with an α-configuration

of this group is more potent in the cat test, and less potent in the frog test, than thevetin, which has a β-configuration.[*]

The sugar moiety affects the absorbability of the glycosides from the intestine and determines the duration of the action. Those glycosides that are easily desugared *in vitro* are generally not satisfactory for medicinal use.

The Emetic Action. The minimum amount of glycoside or poison per kilogram of cat necessary to produce vomiting when given intravenously is called the emetic dose. It is uncertain how the drugs produce this response, but the action does not take place on the vomiting center of the brain.[†] As the data of Table VI show, the correlation between cardiatonic potency and the emetic dose is poor, although qualitatively the order of the drugs in the two effects is nearly the same.

THE DIGITALIS SAPOGENINS

The saponins are a group of glycosides with the ability to produce stable foams when their aqueous solutions are shaken. The cardiac glycosides also produce foams and are saponins, but, because of their characteristic heart action, they are treated as a separate class. The saponins occurring with the cardiac glycosides of the digitalis group are differentiated from the others by he designation "the digitalis saponins."[‡] This separation is chemically correct, for the digitalis saponins contain the cyclopentanoperhydrophenanthrene nucleus and yield Diels' hydrocarbon when dehydrogenated with selenium, whereas most of the other saponins are built up on some other ring system and yield 1,2,7-trimethylnaphthalene (sapotalene) when dehydrogenated.[377]

Like the cardiac glycosides the digitalis saponins taste bitter and are irritating to the mucous membranes. Given intravenously they are poisonous, but taken orally they are non-toxic, probably because they are not absorbed in the intestine. The poisonous properties of these

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[*] Data reported in 1941 supplement these statements. Chen and Elderfield, *J. Pharmacol.*, **70**, 338 (1940), report strophanthidin one-fifth as active in cats and one-third as active in frogs as cymarin; this shows the decrease in potency due to removal of the sugar residue. In addition, a large number of strophanthidin derivatives were examined and found to be nearly inactive. DeGraff, Paff, and Lehmann, *ibid.*, **72**, 211 (1941), have studied the effect of fifteen cardiac glycosides and genins on the embryonic chick heart. This work shows that a variation in the substituents on the nucleus causes profound changes in activity; that attachment of desoxy sugars at the C_3—OH group greatly enhances the activity of the genins; and that attachment of glucose, in addition to the desoxy sugars, usually decreases the potency.

[†] For a critical discussion see Weese, "Digitalis," Thieme, Leipzig (1936).

[‡] For distribution of the saponins see Kofler, "Die Saponine," Springer, Vienna (1927).

[377] *Cf.* Haworth, *Ann. Repts. Chem. Soc. (London)*, **34**, 327 (1937); Jacobs and Elderfield, *Ann. Rev. Biochem.*, **7**, 449 (1938).

glycosides are more pronounced toward lower forms of animal life than higher. For this reason crude extracts of the saponins have been used by primitive peoples to catch fish. The extracts are poured into streams and the fish are either stunned or killed by the glycoside. Since the saponins are not harmful when taken internally, fish killed in this way are edible. One of the most interesting characteristics of the saponins is their ability to hemolyze red blood corpuscles in very low concentration. The dilution (or index) for these various physiological effects is of the same order of magnitude; the dilutions for digitonin are given below:

| Taste index [378] | 1 : 380,000 | Fish index [378, 380] | 1 : 200,000 |
| Eye index [378, 379] | 1 : 230,000 | Hemolytic index [381] | 1 : 168,000 |

The digitalis saponins form solid molecular compounds with the higher alcohols, the phenols, and the thiophenols.[382] The addition compounds formed with digitonin have been studied more thoroughly than those formed with the other saponins, and in all cases the ratio of the saponin to alcohol, or phenol, is 1 : 1. The addition compounds are insoluble in water but are usually soluble in alcohol. Among the neutral steroid alcohols, however, addition compounds that are insoluble in alcohol are formed with the 3(β)-hydroxysteroids and occasionally with the 17(α)-hydroxysteroids.[383] In testing for structure, both members of an epimeric pair should be examined.[384] As a test for configuration at C_3, faulty results may be obtained if the side chain differs greatly from the normal,[385] if the molecule is polyhydric,[385] or if an acetyl group is present at C_{17} and rings A/B have the *allo* configuration.[386] An *epi* configuration of a methyl group at C_{10} prevents formation of an insoluble digitonide even when the steric configuration of the C_3—OH is β (*cf.* lumisterol and pyrocalciferol). When a monomolecular layer of a β- or of an α-sterol is treated with digitonin, the saponin is adsorbed as a visible film

[378] Kofler and Schrutka, *Biochem. Z.*, **159**, 327 (1925).

[379] Kobert, *Arch. exptl. Path. Pharmakol.*, **23**, 257 (1887).

[380] Kofler, *Biochem. Z.*, **129**, 64 (1922). The fish index is usually defined as that dilution required to kill fish weighing 0.1–0.5 g. A species of minnow (Rotauge) is used for the assay.

[381] See Kofler, "Die Saponine." The value given is for human blood.

[382] Windaus, *Ber.*, **42**, 238 (1909); Windaus and Weinhold, *Z. physiol. Chem.*, **126**, 299 (1923).

[383] Schoenheimer and Evans, Jr., *J. Biol. Chem.*, **114**, 567 (1936); Reichstein, *Helv. Chim. Acta*, **19**, 406 (1936); Stoll, *Z. physiol. Chem.*, **246**, 1 (1937); Wintersteiner, *J. Am. Chem. Soc.*, **59**, 765 (1937); Ruzicka, Furter, and Goldberg, *Helv. Chim. Acta*, **22**, 498 (1938).

[384] Noller, *J. Am. Chem. Soc.*, **61**, 2717 (1939).

[385] Fernholz, *Z. physiol. Chem.*, **232**, 97 (1935).

[386] Butenandt and Mamoli, *Ber.*, **68**, 1847 (1935).

by the normal sterol, but only slightly by the *episterol*. The former is more stable than the latter. The digitonides of the β-sterols are hydrophilic, but those of the α-sterols are hydrophobic.[287] The molecular addition products may be broken up with regeneration of the steroids by treating them with pyridine,[288] by extracting with boiling xylene, or by acetylating.[289]

The Digitalis Saponins. The principal members of the digitalis saponins are shown in Table VII. Preparation of these glycosides in pure form is a tedious and uncertain process. From the crude extracts

TABLE VII

PRINCIPAL DIGITALIS SAPONINS *

Saponin	Probable Formula	Plant Source	Hydrolytic Products	
			Sapogenin	Sugars
Trillin	$C_{32}H_{52}O_8$	*Trillium erectum*	Diosgenin	1 Glucose
Trillarin	$C_{39}H_{62}O_{18}$(?)	*Trillium erectum*	Diosgenin	2 Glucose
Sarsasaponin (parillin)	$C_{45}H_{74}O_{17}$	*Radix sarsaparillae*	Sarsasapogenin (parigenin)	2 Glucose and 1 rhamnose
Gitonin	$C_{51}H_{82}O_{23}$	*Digitalis purpurea*	Gitogenin	3 Galactose and 1 pentose
Digitonin	$C_{56}H_{92}O_{29}$	*Digitalis purpurea*	Digitogenin	4 Galactose and 1 xylose
Tigonin	$C_{56}H_{92}O_{27}$	*Digitalis purpurea,* *Digitalis lanata*	Tigogenin	2 Glucose, 2 galactose, and rhamnose
Amolonin	$C_{63}H_{104}O_{31}$	*Chlorogalum pomeridianum*	Tigogenin	3 Glucose, 1 galactose, and 2 rhamnose
Dioscin	$(C_{20}H_{34}O_8)_x$	*Dioscorea tokoro* (Makino)	Diosgenin	Rhamnose (?)

* Data from Tschesche, *Ergeb. Physiol.*, **38**, 65 (1936); Tsukamoto and Ueno, *J. Pharm. Soc., Japan,* **56**, 802 (1936) [*C. A.*, **32**, 7470 (1938)]; Grove, Jenkins, and Thompson, *J. Am. Pharm. Assoc.*, **27**, 457 (1938); Marker and Krueger, *J. Am. Chem. Soc.*, **62**, 3349 (1940).

of the leaves and seeds of the digitalis family, the cardiac glycosides can be removed by means of chloroform or ether. The separation of digitonin from the mixture of digitonin, gitonin, tigonin, and other saponins obtained from *D. purpurea* illustrates the procedures employed. By the

[287] Langmuir and co-workers, *J. Am. Chem. Soc.*, **59**, 1405 (1937).

[288] Schoenheimer and Dam, *Z. physiol. Chem.*, **215**, 59 (1933); Bergmann, *J. Biol. Chem.*, **132**, 471 (1940).

[289] Windaus, *Z. physiol. Chem.*, **65**, 110 (1910).

method of Kiliani [390] the digitonin is precipitated from an aqueous solution as the amyl alcohol addition product, regenerated by removal of the amyl alcohol, and the product recrystallized first from 50 per cent and then from 85 per cent alcohol. Windaus and Shah [391] precipitate the digitonin from a 5 per cent aqueous solution by the addition of ether, with which it forms an addition compound. The precipitate is filtered off at the end of thirty minutes; repetition of the process gives pure digitonin. By the last procedure gitonin is also obtained, since it precipitates with ether on standing.

It is very difficult to separate tigonin from the mixtures of gitonin and tigonin that occur in nature. Fortunately the leaves of *D. lanata* contain only tigonin, and the saponin is easily purified through its sparingly soluble cholesterol addition product. [392]

Recently members of the lily family have been extensively studied as sources of the saponins. [393] From the plants examined, trillin and trillarin have been isolated from *Trillium erectum;* [393] sarsasaponin from the Mexican sarsaparilla root, *Radix sarsaparillae;* [394] amolonin from the California soap plant, or amole; [395] and dioscin from *Dioscorea tokoro* (Makino). [396] The occurrence of these saponins in plants other than the digitalis groups shows that the designation "the digitalis saponins" is not ideal.

The Sapogenins. By acid hydrolysis the saponins are split to sugars and sapogenins. The hydrolytic products are given in Table VII, and, as the list shows, the sugars are not unique. Because of the active interest in the chemistry of the sapogenins, various procedures have been developed for the direct isolation of the aglucons without obtaining the saponins as intermediate products. [397]

Two general structures (I and II) have been proposed for the sapogenins. Both show that they are comparable to the sterols, except that the C_{17} side chain is made up of two heterocyclic oxygen rings. In formula I of Tschesche and Hagedorn, [398] the side chain is composed of two

[390] Kiliani, *Ber.*, **43**, 3562 (1910); **49**, 701 (1916). A summary of the method is given by Lettré and Inhoffen, "Über Sterine, Gallensäuren und verwandte Naturstoffe," Enke, Stuttgart (1936), p. 189.

[391] Windaus and Shah, *Z. physiol. Chem.*, **151**, 86 (1926); *cf.* Windaus and Schneckenburger, *Ber.*, **46**, 2628 (1913).

[392] Tschesche, *Ber.*, **69**, 1665 (1936).

[393] Marker and co-workers, *J. Am. Chem. Soc.*, **62**, 2542, 2548, 2620, 3349 (1940).

[394] Jacobs and Simpson, *J. Biol. Chem.*, **105**, 501 (1934).

[395] Jurs and Noller, *J. Am. Chem. Soc.*, **58**, 1251 (1936); *cf.* Liang and Noller, *ibid.*, **57**, 525 (1935).

[396] Tsukamoto and Ueno, *J. Pharm. Soc. Japan*, **56**, 802 (1936) [*C. A.*, **32**, 7470 (1938)].

[397] *Inter al.*, Fieser and Jacobsen, *J. Am. Chem. Soc.*, **60**, 28 (1938); Noller, Goodson, and Synerholm, *ibid.*, **61**, 1707 (1939); Marker, reference 393.

[398] Tschesche and Hagedorn, **68**, 1412 (1935); **69**, 797 (1936).

TABLE VIII

PRINCIPAL DIGITALIS SAPOGENINS *

Sapogenin	Structural Variations of Sapogenins			Formula	M.P. °C.	$[\alpha]_D$ (CHCl₃)	Source
	Position of OH Groups	Rings A/B	Side Chain				
Monohydroxy Sapogenins							
Diosgenin	3(β)	†	iso	$C_{27}H_{42}O_2$	204-207	−129.3° (CHCl₃?)	Diosein, trillin
Sarsasapogenin	3(β)	cis	normal	$C_{27}H_{44}O_3$	197-198	−75	Sarsasaponin
Episarsasapogenin	3(α)	cis	normal	$C_{27}H_{44}O_3$	204-206	−71	Sarsasapogenone
Tigogenin	3(β)	trans	iso	$C_{27}H_{44}O_3$	204	−49 (pyridine)	Tigonin
Smilagenin	3(β)	cis	iso	$C_{27}H_{44}O_3$	183-184	−69	Jamaica sarsaparilla root
Neotigogenin	3(β)	trans	normal	$C_{27}H_{44}O_3$	202-203	−65	Chlorogalum pomeridianum
Nitogenin	(?)	(?)	(?)	$C_{27}H_{44}O_3$	201	−112	Balantes aegyptica
Dihydroxy Sapogenins							
Gitogenin	2, 3(β)	trans	iso (?)	$C_{27}H_{44}O_4$	272	−61 (dioxane)	Gitonin
Chlorogenin	3(β), 6(α) ‡	trans	iso	$C_{27}H_{44}O_4$	276	−46 (dioxane)	Chlorogalum pomeridianum
Lilligenin	3(β), (?)	(?)	(?)	$C_{27}H_{44}O_4$	245-246	. . .	Lillium rubrum magnificum
Trihydroxy Sapogenins							
Digitogenin	2, 3(β), (?)	trans	iso (?)	$C_{27}H_{44}O_5$	280-283	−81	Digitonin

* Data from Tschesche, *Ber.*, **68**, 1090 (1935); Tschesche and Hagedorn, *Ber.*, **68**, 2247 (1935); Tsukamoto and Ueno, *J. Pharm. Soc. Japan*, **56**, 802 (1936) [*C. A.* **32**, 7470 (1938)]; Askew, Farmer, and Kon, *J. Chem. Soc.*, 1399 (1936); Kon and Weller, *ibid.*, 800 (1939); Noller and co-workers, *J. Am. Chem. Soc.*, **61**, 1707, 2420 (1939); Marker and co-workers, *ibid.*, **62**, 2620 (1940).

† Unsaturated at C₅: C₆.

‡ Noller, footnote p. 1465, questions the position of this hydroxyl group.

tetrahydrofuran rings, one of which is fused with ring D. In formula II of Marker and Rohrmann,[399] the side chain consists of a ketospiroacetal containing a six-membered oxygen ring, and the same fused tetrahydrofuran ring as in I. The evidence for these two proposals is discussed later. Whatever its structure, the side chain may have a "normal" or an "iso" configuration, and by suitable treatment one form may be converted to the other. Of the naturally occurring sapogenins, about half have an *allo* configuration of rings A/B, and all have a 3(β)-hydroxyl group. The structural characteristics and the physical properties of the principal sapogenins are given in Table VIII.

I. Tschesche-Hagedorn   II. Marker-Rohrmann

Proposed structures of the Digitalis Sapogenins
(Rings A/B: *cis* or *trans*)
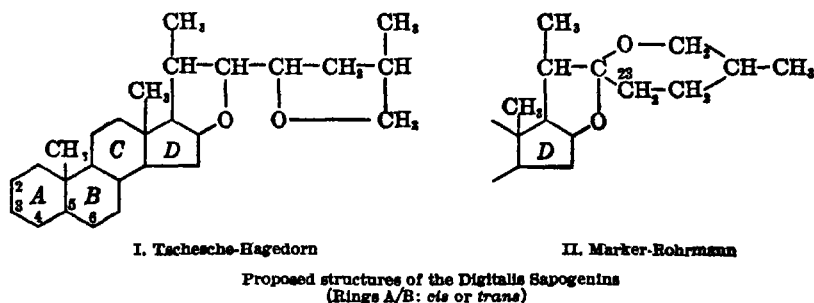
Although the sapogenins are easily purified through their acetyl compounds, their composition as C_{27} steroids was not established until 1935, since the early analyses did not differentiate between C_{26} and C_{27}. It remained for Jacobs and Simpson [400] to alter the accepted compositions, and, at the same time, to dehydrogenate gitogenin and sarsasapogenin with selenium to Diels' hydrocarbon.* With these indications of the nature of the problem, the structure of the nucleus was soon established.

The Ring Nucleus. The structure of the nucleus has come largely from the study of the products obtained in the degradation of the monohydric sapogenins, tigogenin [398] and sarsasapogenin,[401] to etio*allo*bilianic and etiobilianic acids, respectively. For the sake of integration with the rest of the discussion, the transformation with sarsasapogenin, rather than with tigogenin (iso*allo*sarsasapogenin), is shown in formulas III–VIII. By chromic acid oxidation of acetylsarsasapogenin (III), an acetylated

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[399] Marker and Rohrmann, *J. Am. Chem. Soc.*, **61**, 2072 (1939).

[400] Jacobs and Simpson, *ibid.*, **56**, 1424 (1934); *J. Biol. Chem.*, **105**, 501 (1934).

* Another product of selenium dehydrogenation is a hexyl methyl ketone. The same or a similar ketone is obtained when the sapogenins are treated with hydrogen chloride in acetic acid [Ruzicka and van Veen, *Z. physiol. Chem.*, **184**, 69 (1929); Simpson and Jacobs, *J. Biol. Chem.*, **109**, 573 (1935); Fieser and Jacobsen, *J. Am. Chem. Soc.*, **60**, 28 (1938)].

[401] Askew, Farmer, and Kon, *J. Chem. Soc.*, 1399 (1936); Farmer and Kon, *ibid.*, 414 (1937).

lactone (IV) was obtained. After deacetylation and removal of the C_3—OH group, the lactone V was converted by Barbier-Wieland degradation through the compound of structure VI to a mixture of etiobilianic acid (VII) and the lactone of probable structure VIII. Through this degradation the relationship of rings A/B is shown to be *cis* in sarsasapogenin, the attachment of the principal side chain at C_{17} is established, and one of the ether oxygens is placed at C_{16}, extending to form a bridge to C_{22}. This last follows from the behavior of the lactone ring of structure V. It is definitely five-membered, since it is opened with difficulty by alkali in the cold, and the hydroxy acid formed reverts immediately to the lactone form in the presence of acid.

III. Acetylsarsasapogenin   IV   V

VI   VII   VIII
Etiobilianic acid
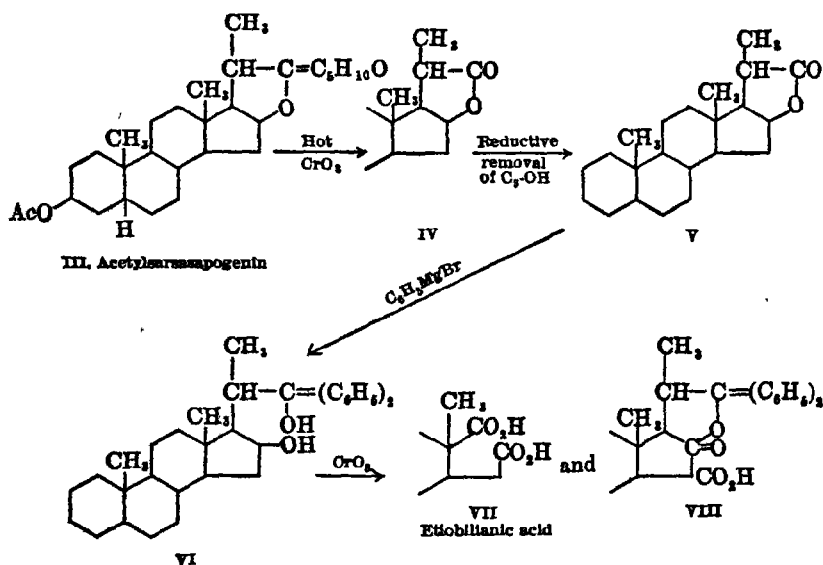
The C_3—OH Group. The attachment of an hydroxyl group at C_3 in tigogenin was first shown in the classical manner by opening the ring bearing the hydroxyl group and subjecting the dicarboxylic acid formed to thermal decomposition. A pyroketone was obtained, and this, taken with the fact that tigogenin forms an insoluble digitonide, placed the hydroxyl group at C_3 in a β-configuration.[402] Subsequently, by means of the digitonide test,[403] by degrading sarsasapogenin and dihydrosarsasapogenin to the known 3(β)-hydroxyetiobilianic acids [404] by converting various sapogenins to known 3(β)-hydroxy derivatives of the sex hor-

[402] Tschesche and Hagedorn, *Ber.*, **68**, 2247 (1935).
[403] Marker and Rohrmann, *J. Am. Chem. Soc.*, **61**, 2724 (1939).
[404] Marker and Rohrmann, *ibid.*, **61**, 2722, 3477 (1939); **62**, 76 (1940).

mones,[405] and by interrelation of the sapogenins, a β-configuration of the C₃—OH group has been found to be a common characteristic of all the natural sapogenins.

The C_{17} Side Chain.   Of the two formulations of the C_{17} side chain, that of Tschesche and Hagedorn was offered to account for the resistance of the side chain to oxidizing agents, and for the production of methyl-succinic acid as one of the products of oxidation.   It was known from
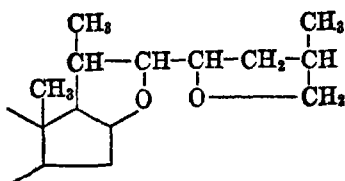
Iα. Sapogenin side chain
(Tschesche-Hagedorn)
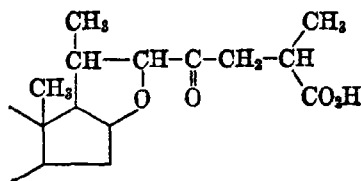
IX. Sapogenoic acid
(Tschesche-Hagedorn)
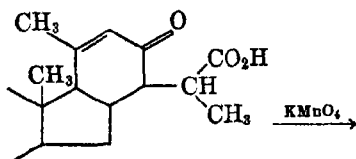
X. Anhydrosapogenoic acid
(Fieser-Jacobsen)
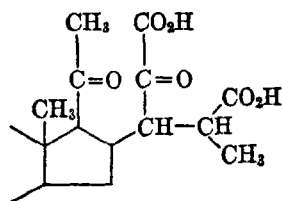
XI. Diketodicarboxylic acid
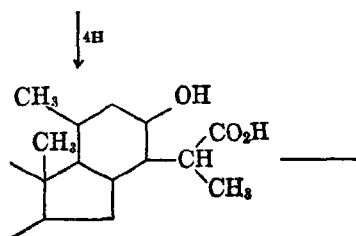
XII. Tetrahydroanhydrosapogenoic acid
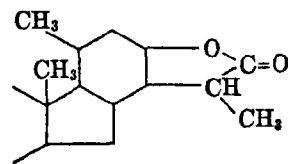
XIII. Lactone

earlier work that vigorous chromic acid oxidation of digitogenin (p. 1466) gave, among other products, methylsuccinic acid and α-methylglutaric acid,[406] or α-methylglutaric acid alone.[407]   Tschesche viewed the α-methylglutaric acid as originating from the nucleus, as is known with the bile acids (p. 1366), and the methylsuccinic acid as arising from the

[405] Marker et al., ibid., 62, 518, 898, 2621, 3003 (1940).
[406] Kiliani, Ber., 49, 702 (1916); 51, 1626 (1918); cf. Windaus, reference 407, p. 47.
[407] Windaus and Willerding, Z. physiol. Chem., 143, 33 (1925).

side chain through the stage of a sapogenoic acid formulated as IX. Fieser and Jacobsen [408] concurred in these formulations after studying various transformations of sarsasapogenoic acid. This acid does not react with ketone reagents under ordinary conditions, but at 130° both the acid and its methyl ester form dioximes (?). It is difficult to hydrogenate the sapogenoic acids, but eventually in acetic acid with Adams catalyst a small proportion is reduced. Heating the acid with dilute alkali, however, converts sarsasapogenoic acid to an anhydro acid (X) formulated as shown. The anhydro acid contains the system C=C—C=O (absorption at 240 mμ) and forms a monoöxime. Oxidation of anhydro-sarsasapogenoic acid with permanganate converts it to a diketodicar-boxylic acid (XI), while hydrogenation reduces the conjugated system to a product (XII) which readily lactonizes (XIII). These formulations seemed consistent, but a number of by-products were obtained which could not be integrated with the other facts, largely because so little was obtained that study was impossible.

Marker and Rohrmann formulated the side chain as a ketospiroacetal (IIa), which allows for the formation of both methylsuccinic acid and α-methylglutaric acid from the side chain through the stage of the sapogenoic acid (XIV).[409] Their principal argument, however, does not rest on the origin of these fragments, but on the lability of the side chain in acid media. It was noted by Fieser and Jacobsen that sarsasapogenone, the 3-ketone corresponding to sarsasapogenin, is isomerized by treatment with acids. On studying other reactions in acid media, Marker and Rohrmann found that the sapogenins are isomerized on prolonged heating in alcoholic hydrochloric acid, are converted to pseudosapogenins by heating with acetic anhydride at 200°, are reduced catalytically in acid media to dihydro derivatives, are brominated easily in the side chain, and are altered by Clemmensen reduction. These changes have been interpreted in the light of the formula proposed by them. On refluxing the sapogenins with a normal configuration of the side chain in alcoholic hydrochloric acid for four days, isomerization occurs in the side chain with the production of isosapogenins (XV).[399] By heating either form with acetic anhydride at 200°, a pseudosapogenin (XVI) is formed which has entirely different properties from the parent sapogenin.[410] In particular, the pseudosapogenins are readily oxidized. Treatment of the pseudosapogenins with alcoholic hydrochloric acid causes a reversion to the sapogenin type if the configuration of rings A/B is cis, to the isosapogenin if this configuration is trans.[411] On oxidation the pseudosapogenins

[408] Fieser and Jacobsen, J. Am. Chem. Soc., 60, 28, 2753, 2761 (1938).

[409] Marker and Rohrmann, ibid., 61, 2072 (1939).

[410] Marker and Rohrmann, ibid., 62, 521 (1940).

[411] Marker and Rohrmann, ibid., 62, 896 (1940).

are attacked at the C_{20}—C_{22} linkage and are converted in good yield to pregnane (p. 1491) derivatives.[410] In acid media catalytic hydrogenation opens the side chain on both the sapogenins and the isosapogenins. The product (XVII) is not identical with that formed by the reduction of the pseudosapogenins (XVIII). According to Marker and Rohrmann,

IIa. Sapogenin side chain
(Marker-Rohrmann)

XIV. Sapogenoic acid

XV. Isosapogenin

XVI. Pseudosapogenin

XVII. Dihydrosapogenin

XVIII. Dihydropseudosapogenin
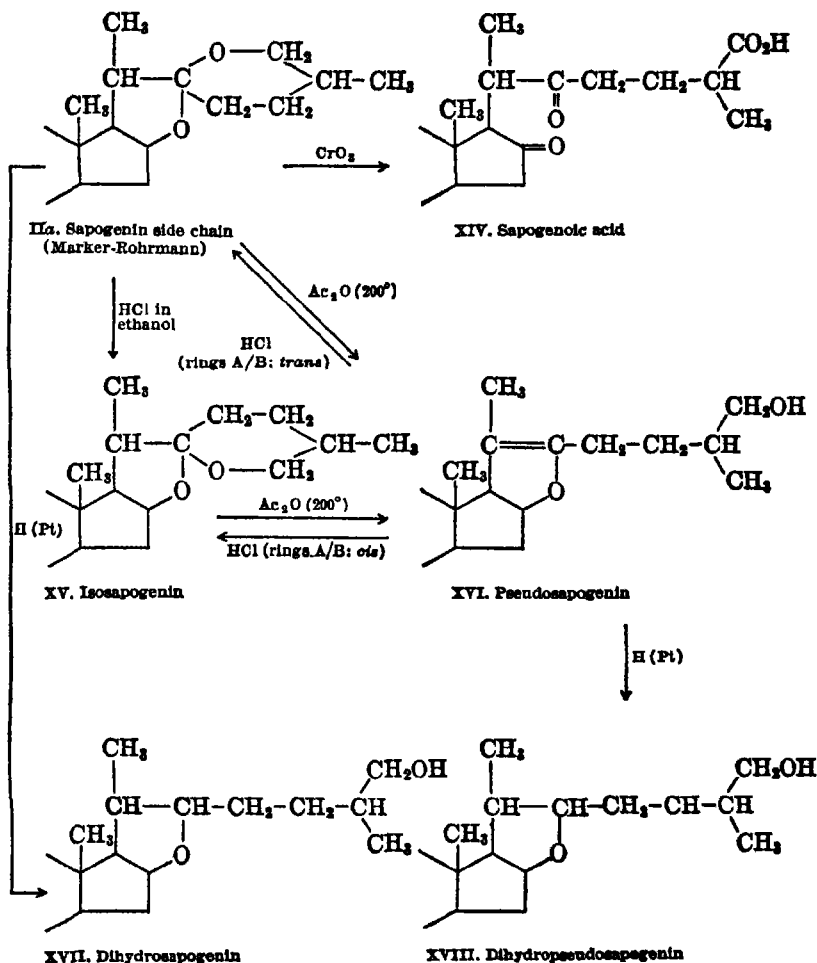
the side chain contains one reactive hydrogen which may be provisonally placed at C_{23}. This hydrogen may be replaced with bromine [412] or brought into reaction with Grignard reagents.[413] Clemmensen reduction opens both oxygen rings with the formation of a tetrahydrosapogenin.[399]

[412] Marker and Rohrmann, *ibid.*, **61**, 1921 (1939); Marker *et al.*, *ibid.*, **63**, 1032 (1941).
[413] Marker and Rohrmann, *ibid.*, **62**, 900 (1940).

Subsequent developments have favored the Marker-Rohrmann formulation, and only synthetic evidence is lacking for rigid proof. Thus, Marker [413a] has isolated α-methylglutaric acid and Δ^{16}-allopregnene-3,20-dione (p. 1494) in good yield by cold chromic acid oxidation of pseudotigogenin and of pseudosarsasapogenin, has obtained 3(β)-hydroxy-16-ketobisnorcholanic acid by ozonolysis of anhydrosarsasapogenoic acid, and has isolated 3(β)-hydroxyetiobilianic acid in poor yield as the product of a double haloform reaction on the ketodicarboxylic acid obtained by permanganate oxidation of anhydrosarsasapogenoic acid. Further, Ladenburg and Noller [413b] have shown that treatment of methyl chlorogenoate diacetate with ammonia gives a product whose ultraviolet absorption spectrum indicates a pyrrole nucleus; this is consistent only with the presence of a 1,4-diketone grouping in the C_{17} side chain.

The Monohydroxysapogenins. The structures of the monohydroxysapogenins have been determined largely by using sarsasapogenin and tigogenin as reference compounds. Diosgenin (XIX), from the saponin dioscin, corresponds in nuclear structure to cholesterol. It gives the reactions characteristic of Δ^5-unsaturation, and on catalytic hydrogenation in neutral media is converted to tigogenin.[414] Episarsasapogenin is not found in nature, but is readily obtained from sarsasapogenin, either by epimerization or by reduction of the corresponding ketone.[415] Smilagenin (isosarsasapogenin) is formed by acid isomerization of the C_{17} side chain of sarsasapogenin.[416] Tigogenin has been obtained from isosarsasapogenin by brominating the corresponding ketone to a 4,23(?)-dibromoketone, debrominating with zinc (Δ^4-ethenoid linkage), and reducing with sodium and ethanol.[417] Application of the same procedure to sarsasapogenin yields neotigogenin.[418] Nitogenin, a sapogenin obtained from an Egyptian date, has not been investigated structurally.[419]

[413a] Marker et al., ibid., **63**, 779, 2274 (1941); Marker and Shabica, ibid., **64**, 180 (1942).
[413b] Ladenburg and Noller, ibid., **63**, 1240 (1941).
[414] Tsukamoto and Ueno, J. Pharm. Soc. Japan, **56**, 802 (1936) [C. A., **32**, 7470 (1938)]; Tsukamoto, Ueno, and Ohta, ibid., **56**, 931 (1936) [C. A., **31**, 3493 (1937)]; **57**, 9 (1937) [Chem. Zentr., (I) 4238 (1937)]; Tsukamoto, Ueno, Ohta, and Tschesche, ibid., **57**, 383 (1937) [Chem. Zentr., (II) 2753 (1938)]; Marker, Tsukamoto, and Turner, J. Am. Chem. Soc., **62**, 2525 (1940); Marker, Jones, and Turner, ibid., **62**, 2537 (1940).
[415] Askew, Farmer, and Kon, J. Chem. Soc., 1399 (1936); Marker and Rohrmann, J. Am. Chem. Soc., **61**, 943 (1939).
[416] Farmer and Kon, J. Chem. Soc., 414 (1937); Kon, Soper, and Woolman, ibid., 1201 (1939); Marker, Tsukamoto, and Turner, J. Am. Chem. Soc., **62**, 2525 (1940).
[417] Marker and Rohrmann, ibid., **61**, 1291, 1516 (1939); Marker, Rohrmann, and Jones, ibid., **62**, 1102 (1940).
[418] Marker and Rohrmann, ibid., **62**, 647 (1940).
[419] Kon and Weller, J. Chem. Soc., 800 (1939).

The Dihydroxysapogenins. Chronologically, the dihydroxysa-pogenin gitogenin was one of the first to be studied. The two hydroxyl groups have been placed at C_2 and C_3 by correlation with tigogenin. When gitogenin is oxidized with cold chromic acid, ring A is opened and a dicarboxylic acid, gitogenic acid, is formed. This acid is identical with one produced by chromic acid oxidation at 70° of tigogenin, by opening ring A.[420] Since rings A/B are *trans* in tigogenin, they must be *trans* in gitogenic acid and in gitogenin. As gitogenic acid must originate by cleavage between C_2 and C_3, this places the hydroxyl groups at the same two positions in the aglucon. Because neither gitogenin nor chlorogenin is isomerized by treatment with alcoholic hydrochloric acid, both are assigned an iso configuration of the C_{17} side chain.[421]

The hydroxyl groups of chlorogenin have been placed at C_3 and C_6* through the application of one of the reactions of cholesterol.[422] Treatment of diosgenin (XIX) with chromic acid at 20° converts it to the corresponding Δ^4-ene-3,6-dione (XX). The double bond of this unsaturated ketone is reduced by zinc and acetic acid to the saturated *allo*-3,6-dione (XXI). Reduction of the latter gives chlorogenin (XXII) if sodium and ethanol are used, and β-chlorogenin (XXIII) if catalytic hydrogenation in alcohol with Adams catalyst is employed. Identical re-

XIX. Diosgenin   XX. Δ^4-Ene-3,6-dione   XXI. 3,6-Dione

XXII. Chlorogenin   XXIII. β-Chlorogenin
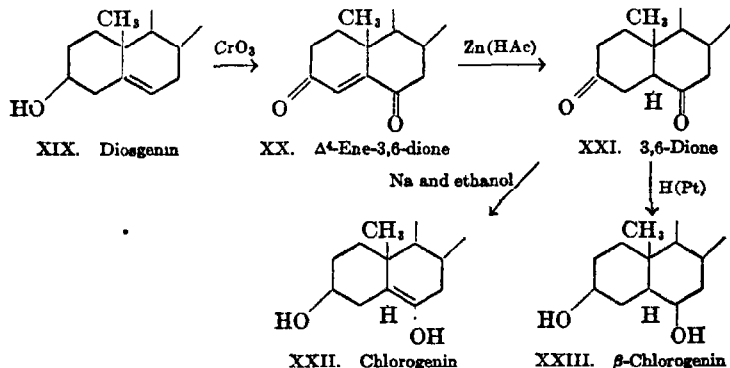
[420] Tschesche, *Ber.*, **68**, 1090 (1935); *cf.* Jacobs and Simpson, *J. Biol. Chem.*, **110**, 429 (1935); Marker and Rohrmann, *J. Am. Chem. Soc.*, **61**, 2724 (1939).

[421] Marker and Rohrmann, *J. Am. Chem. Soc.*, **61**, 2724 (1939); **62**, 647 (1940).

* The position of this hydroxyl group has been questioned by Noller and Lieberman, *J. Am. Chem. Soc.*, **63**, 2131 (1941), on the basis of the following evidence: Chlorogenonic acid, a ketodicarboxylic acid obtained by cold chromic acid oxidation of chlorogenin, is not identical with digitogenic (XXV) or digitoic (XXVIII) acid and is converted by Wolff-Kishner reduction to gitogenic acid. Thus, in the oxidation, cleavage of ring A takes place between C_2 and C_3, and if the second hydroxyl group were attached at C_4 either digitogenic or digitoic acid should result.

[422] Mauthner and Suida, *Monatsh.*, **17**, 579 (1896); Windaus, *Ber.*, **39**, 2249 (1906); **40**, 257 (1907).

sults are obtained if chlorogenone from natural chlorogenin is reduced. Because of this mode of formation, chlorogenin is formulated by Marker as a 3(β),6(α)-dihydroxysapogenin, and β-chlorogenin as a 3(β),6(β)-dihydroxygenin.* In agreement with the assigned *allo* configuration, chlorogenone is not rearranged on treatment with alkali.[423]

Lilligenin has been isolated in such small quantity that an investigation of its structure has not been possible. It appears to have an hydroxyl group adjacent to the one that takes part in digitonide formation.[424]

Digitogenin. When digitogenin (XXIV), which contains three secondary hydroxyl groups, is oxidized with cold chromic acid, one of the products is digitogenic acid (XXV), a ketodicarboxylic acid. On reduction of the carbonyl group of this acid by the Wolff-Kishner method, gitogenic acid is formed.[425] Thus two of the hydroxyl groups of digitogenin are placed at C_2 and C_3, as in gitogenin.[398]

If digitogenic acid is oxidized with permanganate, ring B is opened and a ketotricarboxylic acid (XXVI), "oxydigitogensäure," is formed.[426] This acid is a β-ketonic acid, since it readily loses one or two molecules of carbon dioxide when it is heated. The probable structure of the acid formed by the loss of one molecule of carbon dioxide (and one molecule of water) is shown in structure XXVII. The second molecule of carbon dioxide is split out as indicated by the dotted lines. The formation of acid XXVI shows that the unplaced hydroxyl group is situated near the bridge head of two condensed rings. The position of this hydroxyl group, or the carbonyl produced from it, is further defined by the rearrangement of digitogenic acid to digitoic acid (XXVIII) by warming with alkali. Taking both these reactions into consideration, the third secondary hydroxyl group is placed at C_6.[398] When digitogenic acid is oxidized with chromic acid, the side chain is apparently oxidized

* The results obtained by the two methods of reduction have led Marker, reference 423, to the generalisation that hydrogenation of a 3,6-diketo*allo*steroid with sodium and ethanol results in the production of a 3(β),6(α)-dihydroxy*allo*steroid, while catalytic hydrogenation in neutral media forms a 3(β),6(β)-dihydroxyepimer. Further examples are reported in the cases of reduction products from cholestane-3,6-dione and 3,6-diketo*allo*cholanic acid. It is surprising that small amounts of the other epimers, 3(α),6(α) and 3(α),6(β), have not been encountered. Using the Meerwein-Ponndorf procedure with aluminum isopropoxide, Tukamoto, *J. Biochem. (Japan)*, **32**, 451, 461 (1940), finds that 3,6-diketo*allo*cholanic acid is converted to 3(α),6(α)- and 3(α),6(β)-dihydroxy acids, and that 3,6-diketocholanic acid yields a mixture of the four theoretically possible dihydroxy acids, with 3(α),6(α)-dihydroxyeholanic acid predominating.

[423] Marker, Jones, and Turner, *J. Am. Chem. Soc.*, **62**, 2537 (1940); *cf.* Marker and co-workers, *ibid.*, **61**, 946, 3479 (1939); **62**, 3006, 3009 (1940); **64**, 221, 809 (1942).

[424] Marker and co-workers, *ibid.*, **62**, 2620 (1940).

[425] Tschesche, *Ber.*, **68**, 1090 (1935).

[426] Windaus and Weil, *Z. physiol. Chem.*, **121**, 62 (1922).

to the sapogenoic acid structure and ring B is opened as indicated in structure XXIX.*

XXIV. Digitogenin

XXV. Digitogenic acid

XXVI

Gitogenic acid

XXIX

XXVIII. Digitoic acid

XXVII
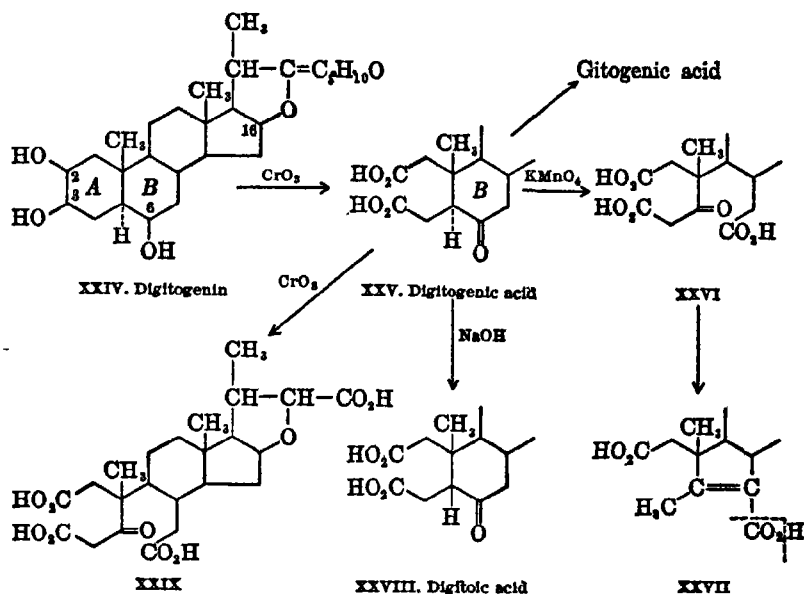
Steroid Alkaloids. Related to the sapogenins are a number of little-investigated steroid alkaloids isolated as glycosides from certain members of the genus *Solanaceae*.[427] The best known of this group are the two glycosides, solanine-*t*, $C_{46}H_{73}O_{15}N$, isolated from potato (*Solanum tuberosum*) sprouts, and solanine-*s*, $C_{45}H_{73}O_{16}N$, obtained from various other *Solanaceae*. According to Rochelmeyer,[428] these are better described as solatunine and solasonine, respectively. On hydrolysis, the aglucons solanidine-*t* (solatubine), $C_{27}H_{43}ON$, and solanidine-*s* (solasodine), $C_{27}H_{43}O_2N$, are formed. Both of these give Diels' hydrocarbon when dehydrogenated with selenium, form insoluble digitonides, contain one double bond, and give the reactions for a tertiary nitrogen. Solanidine-*s*, on distillation with zinc, yields a mixture of pyrrole bases.[428] Solanidine-*t* appears to be structurally similar to cholesterol and may be represented provisionally by either XXX or XXXa.[429]

* Degradations by Marker *et al.*, *J. Am. Chem. Soc.*, **64**, 1843 (1942), show that the third hydroxyl group of digitogenin is not at C_6 and may be at C_{15}.

[427] *Cf.* Schöpf and Herrmann, *Ber.*, **66**, 298 (1933); Oddo and Caronna, *Ber.*, **67**, 440 (1934).

[428] Rochelmeyer, *Arch. Pharm.*, **274**, 543 (1936); **275**, 336 (1937); *Ber.*, **71**, 226 (1938); *Arch. Pharm.*, **277**, 329, 340 (1939).

[429] Soltys and Wallenfels, *Ber.*, **69**, 811 (1936); Clemo, Morgan, and Raper, *J. Chem. Soc.*, 1296 (1936).

Solanidine-s apparently differs from solanidine-t by an additional tertiary hydroxyl group.[430]

XXX. Solanidine-t
(Soltys-Wallenfels)

or

XXXa. Solanidine-t
(Clemo, Morgan, and Raper)
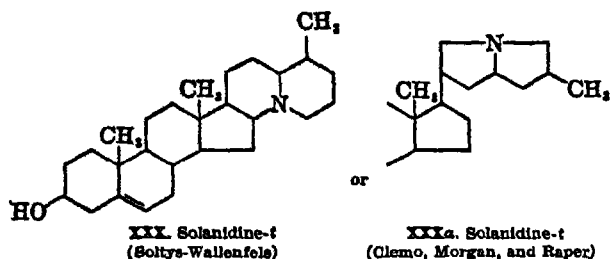
THE SEX HORMONES *

It has been known for years that the glands of the genital systems elaborate substances that cause important physiological changes. By studying the results of castration, and of castration followed by implantation of the glands removed, or of those of the opposite sex, physiologists were able to determine some of the effects due to secretions of the genital glands of the two sexes. A further stage was reached when the effect of extracts of the gonads on castrated animals was studied. Through these methods definite bioassays have been developed for the evaluation of the several hormones. At the present time three types of sex hormones are recognized as originating in the gonads: the estrogenic hormones, the hormone of the corpus luteum, and the androgenic hormones. The structures of the glandular hormones are shown in formulas I–III. α-Estradiol (I) is the ovarian hormone that produces

I. α-Estradiol

II. Progesterone
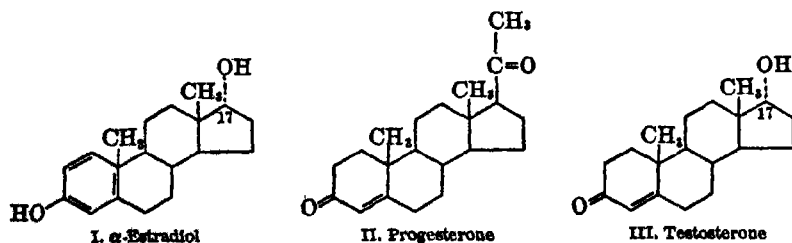
III. Testosterone

estrus; progesterone (II), the corpus luteum hormone that is essential for pregnancy; and testosterone (III), the testicular hormone that causes changes in the accessory sexual organs of the male. The production of these sex hormones appears to be regulated by the gonadotropic

[430] Briggs, *J. Am. Chem. Soc.*, **59**, 1404 (1937); *Nature*, **144**, 247 (1939).

* For physiology see Allen, "Sex and Internal Secretions," 2nd ed., Williams and Wilkins Co., Baltimore (1939).

hormones secreted in the anterior lobe of the pituitary. That these hormones from the pituitary are responsible for the production of the sex hormones has been shown by the removal of the gland and by the injection of extracts.

The Estrogenic Hormones *

In the female sex organs a periodic change takes place which varies somewhat from species to species. As the result of the action of the estrogenic hormones, the female is brought into a state of heat (estrus), during which she will mate. In estrus, rats, mice, and guinea pigs show characteristic changes in the tissues of the vagina, accompanied by a typical vaginal discharge that has a unique cornified appearance. By microscopic examination of the vaginal smears from such an animal the estrous condition is easily recognized. Allen and Doisy,[431] using castrated female rats and mice, adapted this phenomenon to a biological assay of estrogenic activity. At the present time the assay is carried out by injecting subcutaneously into a group of five or more castrated mice (or rats) several concentrations of the substance under examination. A group of control animals is simultaneously injected with a standard estrogenic substance. By comparison of the concentrations necessary to produce estrus in more than 50 per cent of the animals the assay can be made with some precision. The results are generally expressed in mouse units (M. U.); by international agreement one mouse unit is defined as the effect produced by 0.1 μg. of a standard estrone preparation.[432] As a subsidiary standard the monobenzoyl ester of α-estradiol (I) is used; this unit (benzoate unit) is represented by the specific activity contained in 0.1 μg. of the ester.[433]

Occurrence. It was not until the development of the Allen-Doisy vaginal smear technique of studying estrus that sources of the hormone could be examined. Since then the estrogens have been found to be present in the gonads and in the placenta, but these organs have a low hormonal content. The hormones are eliminated in the urine of both sexes and although the content of pregnancy urine is high, the urines of the stallion and other males of the Equidae are the richest sources known. In Table IX representative values of the several urines are given. Estrogenic hormones have been found in the lower forms of animal life and in the plant kingdom. Thus, one of the hormones—

* For biochemistry see Doisy, Chapter XIII, and Gustavson, Chapter XIV, in Allen "Sex and Internal Secretions," 2nd ed., Williams and Wilkins Co., Baltimore (1939).

[431] Allen and Doisy, J. Am. Med. Assoc., 81, 819 (1923).

[432] Lormand, Bull. soc. chim. biol., 15, 1566 (1933).

[433] Gautier, Quart. Bull. Health Organisation League Nations, IV, 543 (1935).

TABLE IX

CONTENT OF ESTROGENIC HORMONE OF VARIOUS URINES *

	Normal		Pregnant	
	M. U. per Liter	M. U. per Diem	M. U. per Liter	M. U. per Diem
Woman........	425	600	21,000	31,000
Man..........	160	240		
Mare.........	200	2,000	100,000	1,000,000
Stallion.......	170,000	1,700,000		
Zebra (male)....	36,000			
Bull..........	330			

* Data from Borchardt, Dingemanse, and Laqueur, *Naturwissenschaften*, **22**, 190 (1934); Zondek, *Nature*, **132**, 209, 494 (1934); *J. Physiol.*, **81**, 472 (1934).

estrone—has been isolated from palm kernel extract,[424] and another—estriol—from pussywillows.[425] Potent extracts have been obtained from a wide variety of sources, including petroleum and coal tar,* but it is uncertain whether the activity is due to true hormones or to other compounds.

Isolation. The procedure used for the isolation and purification of the estrogenic hormones is rather complex, since it requires a concentration of about a millionfold and a separation from substances that are physically and chemically similar. Pregnancy urine of women or of mares, or the urine of stallions, is the usual source. In the urine the hormones are present to some extent as glucuronides,[426] or as sulfates,[427] from which they are liberated by boiling with concentrated hydrochloric acid. The hormones may then be extracted with organic solvents (e.g., n-butanol); the extracts are freed of acidic impurities (auxin a, etc.) and purified to a high degree by partition between various solvents. By an optional procedure, the hormones, after extraction, are caused to react with Girard's reagent T (trimethylaminoacetohydrazide)[428] and

[424] Butenandt and Jacobi, *Z. physiol. Chem.*, **218**, 104 (1933).

[425] Skarzynski, *Nature*, **131**, 766 (1933).

* For list see Table I, Doisy, p. 849–850, in Allen, "Sex and Internal Secretions," 2nd ed., Williams and Wilkins Co., Baltimore (1939).

[426] Cohen and Marrian, *Biochem. J.*, **30**, 57 (1936); Cohen, Marrian, and Odell, *ibid.*, **30**, 2250 (1936); Callow, *ibid.*, **30**, 906 (1936); Odell, Marrian, and Skill, *Am. J. Pharm.*, **99**, 420 (1937).

[427] Schachter and Marrian, *J. Biol. Chem.*, **126**, 663 (1938); Butenandt and Hofstetter, *Z. physiol. Chem.*, **259**, 222 (1939).

[428] Girard and Sandulesco, *Helv. Chim. Acta*, **19**, 1095 (1936).

are converted to water-soluble derivatives that are easily freed from the oils that accompany them at this stage. The final purification is usually carried out by distilling in a high vacuum, followed by recrystallization. Addition compounds, such as quinoline with estrone, or the acid half esters formed with phthalic or succinic anhydrides, have been recommended for the final purification. By other procedures the hormones are adsorbed from the urine and the adsorbate worked up in much the same manner as that portrayed above.*

The natural estrogenic hormones are derivatives of the hypothetical hydrocarbon estrane (IV).[439] All the natural estrogens are phenols with
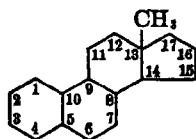
IV. Estrane

the phenolic hydroxyl group at C_3, and with ring A, or rings A and B, benzenoid. In ring D there is an oxygen function at C_{17}, and the nature of this group determines to a large degree the physiological activity. Many of the hormones occur in polymorphic modifications,[440] and nearly all absorb strongly in the ultra-violet at 280–285 mμ.[441] In keeping with their phenolic nature, the estrogens give a number of color reactions, especially with phenol sulfonic acids, that are useful for quantitative estimation.†

Estrone and Estriol. Estrone (VI), the first of the estrogenic hormones to be isolated in pure form, was reported at nearly the same

* Selected literature on isolation: Butenandt and Hildebrandt, Z. physiol. Chem., 199, 243 (1931); Marrian, Biochem. J., 23, 1090, 1233 (1929); 24, 435, 1021 (1930); Curtis, MacCorquodale, Thayer, and Doisy, J. Biol. Chem., 107, 191 (1934); Cartland, Meyer, Miller, and Ruts, ibid., 109, 213 (1935). U. S. pats.: Doisy, Thayer, and Veler, 1,967,350; Doisy, 1,967,351; Butenandt, 2,012,300; Schwenk and Hildebrandt, 2,046,656; Schwenk and Hildebrandt, 2,054,271; Schoeller, Schwenk, and Hildebrandt, 2,103,735; Schwenk and Whitman, 2,174,532; and Schwenk and Hildebrandt, 2,178,109.

439 Adam et al., Nature, 132, 205 (1933). At the suggestion of Professor A. M. Patterson this nomenclature has been modified by including parenthetically the linkage of the double bonds from the bridge heads.

440 Kofler and Hauschild, Z. physiol. Chem., 224, 150 (1934).

441 See Morton, "Absorption Spectra of Vitamins and Hormones," Hilger, London (1935), p. 64; cf. Rowlands and Callow, Biochem. J., 29, 837 (1935).

† Color reactions: Kober, Biochem. Z., 239, 209 (1931); Schwenk and Hildebrandt, ibid., 259, 240 (1933); Haussler, Helv. Chim. Acta, 17, 531 (1934); Zimmermann, Z. physiol. Chem., 233, 257 (1935); Pincus, Wheeler, Young, and Zahl, J. Biol. Chem., 116, 253 (1936); Voss, Z. physiol. Chem., 249, 218 (1937); cf. Marrian, Ergeb. Vitamin-Hormonforsch., 1, 447 (1938), for a discussion of the color tests.

TABLE X

PRINCIPAL ESTROGENIC HORMONES *

Hormones †	Structure	Formula	M.P. °C.	$[\alpha]_D$ (Dioxane)	Physiological Potency	
					μg./M. U.‡	μg./R. U.§
d-Equilenin	3-Hydroxy-17-keto-Δ$^{1,3,5(10),6,8(9)}$-estrapentaene	C$_{18}$H$_{16}$O$_2$	250-251	+ 87°	10-12	30 (15-20)
l-Equilenin	3-Hydroxy-17-keto-Δ$^{1,3,5(10),6,8(9)}$-estrapentaene	C$_{18}$H$_{16}$O$_2$	250-251	- 85		400
d-Isoequilenin	3-Hydroxy-17-keto-Δ$^{1,3,5(10),6,8(9)}$-estrapentaene	C$_{18}$H$_{18}$O$_1$	257-258	+147		> 500
l-Isoequilenin	3-Hydroxy-17-keto-Δ$^{1,3,5(10),6,8(9)}$-estrapentaene	C$_{18}$H$_{18}$O$_2$	257-258	-147		> 500
α-Dihydroequilenin	3,17(α)-Dihydroxy-Δ$^{1,3,5(10),6,8(9)}$-estrapentaene	C$_{18}$H$_{20}$O$_2$	248			10-20
β-Dihydroequilenin	3,17(β)-Dihydroxy-Δ$^{1,3,5(10),6,8(9)}$-estrapentaene	C$_{18}$H$_{20}$O$_2$	215-217 c.	- 4 7	ca. 2-4	
Equilin	3-Hydroxy-17-keto-Δ$^{1,3,5(10),7}$-estratetraene (?)	C$_{18}$H$_{20}$O$_2$	234-240 c.	+308	0.7-0.8	1-1.5
Hippulin		C$_{18}$H$_{20}$O$_2$	233 c.	+128	0.7-0.8	
Estrone	3-Hydroxy-17-keto-Δ$^{1,3,5(10)}$-estratriene	C$_{18}$H$_{22}$O$_2$	259 ‖	+170	0.1	0.8
Folliculosterone	3(β)-Hydroxy-17-keto-Δ$^{5(10),6,8(9)}$-estratriene (?)	C$_{18}$H$_{22}$O$_2$	248	+162 (CHCl$_3$)	(?)	
α-Estradiol	3,17(α)-Dihydroxy-Δ$^{1,3,5(10)}$-estratriene	C$_{18}$H$_{24}$O$_2$	176-178 c.	+ 80	0.02	0.1
β-Estradiol	3,17(β)-Dihydroxy-Δ$^{1,3,5(10)}$-estratriene	C$_{18}$H$_{24}$O$_2$	220-223 c.	+ 54	2-4	
Estriol	3,16,17-Trihydroxy-Δ$^{1,3,5(10)}$-estratriene	C$_{18}$H$_{24}$O$_3$	281 c.	+ 30	ca. 10	

* Data from Doisy, Chapter XIII, in Allen, "Sex and Internal Secretions," 2nd ed., Williams and Wilkins Co., Baltimore (1939); Marrian, *Ergeb. Vitamin-Hormon-forsch.*, 1, 451 (1938); Remesov, *Rec. trav chim.*, 56, 1093 (1937); Inhoffen *et al.*, *Ber.*, 71, 1024 (1938); Marker, *J Am. Chem. Soc*, 60, 1897 (1938); Bachmann *et al.*, *ibid.*, 61, 824 (1940).

† Synonyms: Estrone—follicular hormone, estrin, theelin. Estradiol—dihydrofollicular hormone. Estriol—follicular hormone hydrate, emmenin, theelol.

‡ M. U. = mouse unit.

§ R. U. = rat unit.

‖ M.p. of the rhombic stable form. The rhombic metastable crystals melt at 254°, and the monoclinic metastable at 256°.

time by Doisy [442] and by Butenandt.[443] Soon after this it was obtained by others,[444] and a year later estriol (V) was isolated by Marrian.[445] At first there was little of either hormone, but x-ray and surface-film measurements, taken with other early findings, suggested that the estrogens were similar to the sterols.[446] The problem of their structures was simplified somewhat when it was found that dehydration of estriol with

V. Estriol   VI. Estrone   Chrysene

VII. Phenoldicarboxylic acid   VIII. Dimethylphenanthrol   IX. Dimethylphenanthrene

Etiobilianic acid
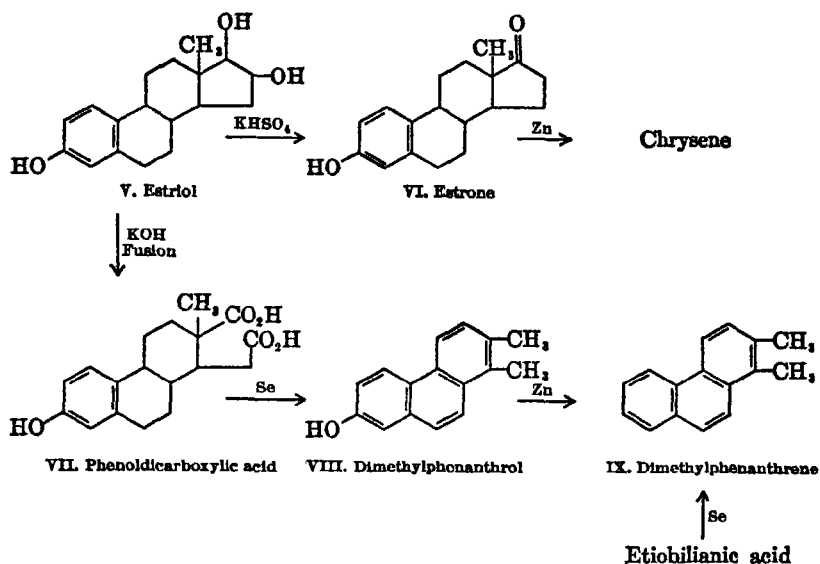
potassium acid sulfate converted it to estrone, thus showing that both compounds have the same nucleus.[447] Although estrone, on distillation with zinc, was converted to chrysene,[448] the structure of the nucleus was established, and the position of the phenolic hydroxyl group was indicated by a transformation of estriol. On fusion with potassium hydroxide, estriol gave a phenolic dicarboxylic acid (VII),[449] which, on selenium

[442] Doisy, Veler, and Thayer, Am. J. Physiol., 90, 329 (1929); J. Biol. Chem., 86, 499 (1930); 87, 357 (1930).

[443] Butenandt, Naturwissenschaften, 17, 879 (1929); Butenandt and v. Ziegner, Z. physiol. Chem., 188, 1 (1930).

[444] D'Amour and Gustavson, J. Pharmacol., 40, 485 (1930); deJongh, Kober, and Laqueur, Biochem. Z., 240, 247 (1931).

[445] Marrian, Biochem. J., 24, 435 (1930).

[446] Bernal, J. Soc. Chem. Ind., 51, 259 (1932); Adam, Danielli, Haslewood, and Marrian, Biochem. J., 26, 1233 (1932); Danielli, Marrian, and Haslewood, ibid., 27, 311 (1933); Danielli, J. Am. Chem. Soc., 56, 746 (1934). The original measurements did not differentiate well between several possible structures and were at first misinterpreted.

[447] Butenandt et al., Z. physiol. Chem., 199, 243 (1931); Marrian and Haslewood, Biochem. J., 26, 25 (1932).

[448] Butenandt and Thompson, Ber., 67, 140 (1934).

[449] Marrian and Haslewood, J. Soc. Chem. Ind., 51, 277T (1932); MacCorquodale, Thayer, and Doisy, J. Biol. Chem., 99, 327 (1933).

dehydrogenation was converted to a dimethylphenanthrol.[450] The latter, on distillation with zinc, gave 1,2-dimethylphenanthrene, which was obtained also when etiobilianic acid (p. 1361) was dehydrogenated with selenium.[450] From this it appeared that the steroid ring system was present in the two estrogens, and, in analogy with the other steroids, the phenolic hydroxyl group was placed at C_3. Proof of the allocation of the hydroxyl group to this position was first obtained by reducing estrone methyl ether (X) to the desoxo ether XI and subjecting this to selenium dehydrogenation.[451] The product, 7-methoxy-1,2-cyclopentenophenanthrene (XII), was isolated in a 1.5 per cent yield, and its structure was established by synthesis. Further evidence for the position of the hydroxyl group was obtained when the dimethylphenanthrol X, formed in the degradation of estriol, was shown by synthesis to have the structure 7-hydroxy-1,2-dimethylphenanthrene.[452] The benzenoid nature of ring A was established by surface-film measurements,[446] by the phenolic properties,[453] and by the uptake of three moles of hydrogen by desoxoestrone on catalytic reduction.[454]
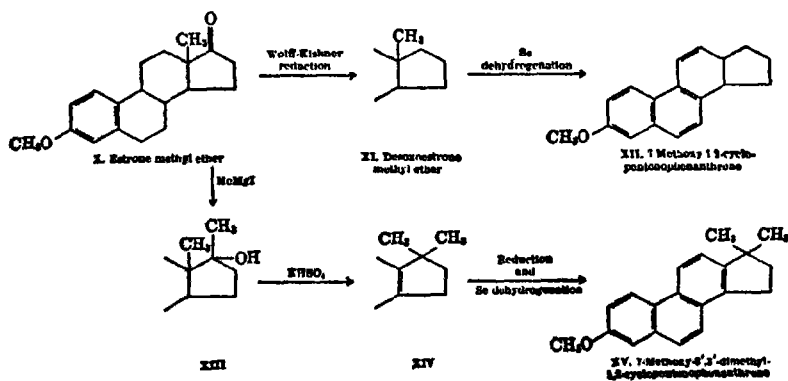
The position of the carbonyl group and of the angular methyl group at C_{13} was established by Cohen, Cook, and Hewett.[455] The methyl ether of estrone was caused to react with methylmagnesium iodide, the resulting carbinol (XIII) was dehydrated to an unsaturated ether (XIV), and this product was subjected first to catalytic reduction and then to selenium dehydrogenation. The product, as was shown by synthesis,

[440] Butenandt, Weidlich, and Thompson, *Ber.*, **66**, 601 (1933).

[451] Cook and Girard, *Nature*, **133**, 377 (1934); Cohen, Cook, Hewett, and Girard, *J. Chem. Soc.*, **653** (1934).

[452] Hawthorn and Sheldrick, *J. Chem. Soc.*, 864 (1934).

[453] *Cf.* Callow, *Biochem. J.*, **30**, 906 (1936).

[454] Butenandt and Westphal, *Z. physiol. Chem.*, **223**, 147 (1934).

[455] Cohen, Cook, and Hewett, *J. Chem. Soc.*, 445 (1935).

proved to be 7-methoxy-3′,3′-dimethyl-1,2-cyclopentenophenanthrene (XV), and not the expected 7-methoxy-3′-methyl-1,2-cyclopenteno-phenanthrene. To explain its formation a molecular rearrangement must be assumed. The carbinol (XIII) resulting from the interaction of methylmagnesium iodide has an hydroxyl group adjacent to a quarternary carbon atom and is of the type in which dehydration should be accompanied by rearrangement. That the angular methyl group actually wanders to C_{17} is shown by the fact that migration occurs when the methyl ether of dihydroestrone is similarly dehydrated, reduced, and dehydrogenated with selenium. The product in this case is 7-methoxy-3′-methyl-1,2-cyclopentenophenanthrene, a hydrocarbon whose structure was established also by synthesis. The migrating methyl group found in the 3′-position in the end product of both these transformations must have been attached originally to the ring system as an angular methyl group. The transformation shows that this methyl group is attached at C_{13} and that the carbonyl group is located at C_{17} in estrone.

The conclusions reached from the study of the degradation products have been confirmed by the conversion of dehydroneoergosterol to estrone and by the total synthesis of equilenin, one of the estrogens present in pregnancy urine of mares. The latter will be considered first.

Total Synthesis of Equilenin. As the structural chemistry of the steroids developed, it became evident that the estrogenic hormones offered one of the best fields for synthetic work since they are relatively simple and easily characterized. Nevertheless, nearly all the synthetic work has led to interesting methods of forming polynuclear hydrocarbons rather than to the sex hormones themselves.[466] In 1939–40, however, Bachmann, Cole, and Wilds [467] developed a synthesis of equilenin starting with 7-methoxy-1-keto-1,2,3,4-tetrahydrophenanthrene (XVI), prepared from 1-naphthylamine-6-sulfonic acid (Cleve's acid). On the tetrahydrophenanthrene system, Bachmann et al. operated first at C_2 of XVI to provide for the future C_{17} carbonyl and C_{13}—CH_3 groups, and then extended a carbon chain from C_1 to develop the remainder of the desired five-membered ring.

Condensation of XVI with methyl oxalate in the presence of sodium methoxide gave the glyoxalate XVII. In the key reaction of the synthesis this glyoxalate was heated at 180° with powdered soft glass, whereupon carbon monoxide was smoothly evolved with the production of the ketonic ester XVIII. The future angular methyl group was then

[466] Reviews: Dane, *Angew. Chem.*, **52**, 655 (1939); Springall, *Ann. Rpts. Chem. Soc (London)*, **36**, 286 (1939).

[467] Bachmann, Cole, and Wilds, *J. Am. Chem. Soc.*, **61**, 974 (1939); **62**, 824 (1940).

introduced by treatment of the sodium derivative of the ketonic ester with methyl iodide. A Reformatsky reaction was carried out on the C-methyl ketonic ester XIX, and the resulting β-hydroxypropionic acid XX was dehydrated by the action of thionyl chloride followed by alcoholic potash. On acidification of the alkaline reaction product, an unsaturated acid (XXI) and an unsaturated acid anhydride were obtained. The unsaturated acid anhydride, or the acid corresponding to it, may be regarded as a *cis* form, and acid XXI as a *trans* form. On reduction with sodium amalgam, both the acid and the anhydride gave a mixture of the two acids, *cis*- and *trans*-7-methoxy-2-methyl-2-carboxy-1,2,3,4-tetrahydrophenanthrene-1-acetic acids (XXII). The stereoisomeric acids were separated by crystallization into an α-acid, m.p. 222–225°, and a β-acid, m.p. 213–214°. Using the β-acid, the acetic acid side chain was lengthened by the method of Arndt-Eistert,[453] and the reaction product XXIV cyclized by heating with sodium methoxide in an atmosphere of nitrogen to 16-carbomethoxy-*dl*-equilenin (XXV). The racemic hormone XXVI was then obtained by hydrolysis with acid followed by decarboxylation and demethylation. Finally, resolution was effected by crystallizing the *l*-menthoxyacetic esters and saponifying. A similar series of reactions on the α-form of acid XXII gave *d*- and *l*-isoequilenins, isomeric at C_{14} with equilenin, and identical with products obtained by the dehydrogenation of isoequilin (p. 1478). In nearly all the eleven steps of the synthesis yields of 90 per cent were obtained, and from 10 grams of 7-methoxy-1-keto-1,2,3,4-tetrahydrophenanthrene about 2.5 grams of *dl*-equilenin and an equal amount of *dl*-isoequilenin were isolated.

Obviously, this synthesis is a classic, not only because of the achievement, but also because of the thorough manner in which it was done. It should be noted that the work of Haworth [459] was helpful in the earlier stages of the synthesis, while that of Cohen, Cook, and Hewett [455] furnished the background for the final stages. Through the synthesis of equilenin, many of the conclusions as to the nature of the steroid nucleus arrived at by degradation are confirmed, since, as is shown below, equilenin has been converted to estrone, and estrone has been prepared from dehydroneoergosterol.*

Estrone from Dehydroneoergosterol. In 1936 Marker [460] reduced dehydroneoergosterol to tetrahydroneoergosterol (XXVII), and by chromic acid oxidation converted the latter to estrone. This result was

[453] Arndt and Eistert, *Ber.*, **68**, 200 (1935); Eistert, *Angew. Chem.*, **54**, 124 (1941).

[459] Haworth, *J. Chem. Soc.*, 1135 (1932).

* A physiologically inactive isomer of estrone, in which rings C/D are probably *cis*, has been prepared by Dane and Schmitt, *Ann.*, **537**, 246 (1939).

[460] Marker, Kamm, Oakwood, and Laucius, *J. Am. Chem. Soc.*, **53**, 1503 (1936).

questioned by Windaus,[461] who found that sodium reduction of dehydro-neoergosterol (p. 1401) leads to the hydrogenation of ring A rather than of ring B. Further study by various workers [462] has shown that sodium

XVI. 7-Methoxy-1-keto-1,2,3,4-tetrahydrophenanthrene   XVII   XVIII

XIX   XX

XXI   XXII

XXIII   XXIV

XXV
16-Carbomethoxy-dl-equilenin   XXVI. dl-Equilenin
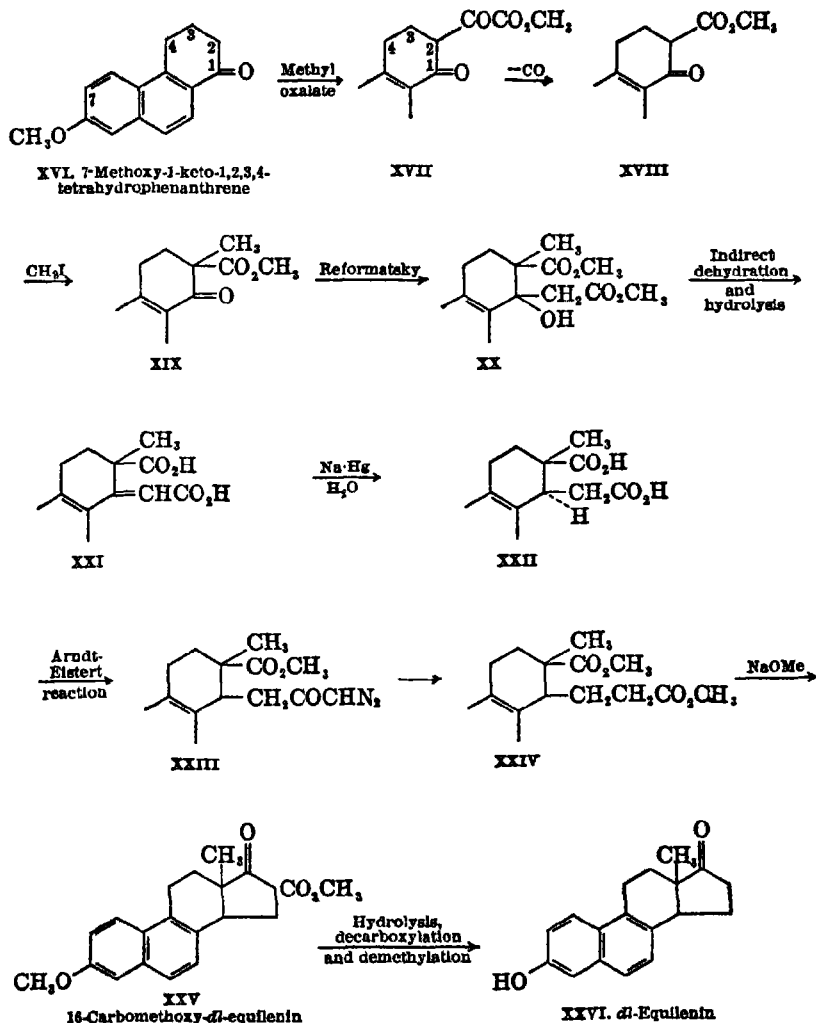
and alcohol reduction of the system present in dehydroneoergosterol and in equilenin gives about 20 per cent phenolic and 80 per cent non-phe-

[461] Windaus and Deppe, Ber., 70, 76 (1937).

[462] Marker, J. Am. Chem. Soc., 60, 1897 (1938); Ruzicka, Müller, and Mörgeli, Helv. Chim. Acta, 21, 1394 (1938); David, Acta Brevia Neerland. Physiol. Pharmacol. Microbiol., 8, 211 (1938) [C. A., 33, 2528 (1939)]; Marker and Rohrmann, J. Am. Chem. Soc., 61, 3314 (1939).

nolis bodies. In the case of reduction of equilenin by this method, estrone or α-estradiol is obtained in about 4 per cent yield. Both Marker and Ruzicka have pointed out that these results are analogous to those obtained in the reduction of β-naphthol with sodium and alcohol.[463]

If the C_{17} side chain of neoergosterol (p. 1401) is converted to carbonyl by ozonization and Barbier-Wieland degradation, the compound known as folliculosterone (XXVIII) is formed.[464] This substance was
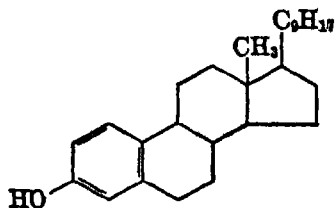
XXVII.
Tetrahydrodehydroneoergosterol
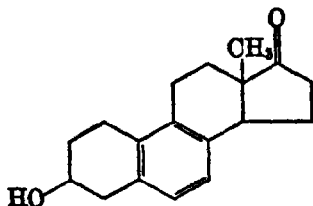
XXVIII. Folliculosterone
(provisional)

reported by Remesov to have a physiological activity about equal to that of estrone, in spite of the fact that ring B is benzenoid rather than ring A. Ruzicka,[462] however, has prepared the two C_3 epimers of the corresponding dihydro compounds and has found that both are weakly estrogenic. In view of the fact that the C_{17} dihydro compounds are usually much more potent than the corresponding C_{17} ketones, the results of the two investigators are contradictory.

Equilin. In the urine of pregnant mares estrone and equilenin are predominantly present, but there are also small amounts of equilin,[465] hippulin,[466] and 17(α)-dihydroequilenin.[466] Girard[465] has noted that, during pregnancy in mares, first estrone, then equilin, and finally equilenin are excreted in increasingly larger proportions. Hippulin has not been studied adequately, but the structure of equilin (XXIX), which

[463] Bamberger and Kitschelt, *Ber.*, **23**, 885 (1890).

[464] Remesov, *Rec. trav. chim.*, **55**, 797 (1936); **56**, 1092 (1937).

[465] Girard *et al.*, *Compt. rend.*, **194**, 909, 1020 (1932); **195**, 981 (1932); *Compt. rend. soc. biol.*, **112**, 964 (1933); Cartland and Meyer, *J. Biol. Chem.*, **112**, 9 (1935).

[466] Winterstein *et al.*, *J. Am. Chem. Soc.*, **58**, 2652 (1936); Marker *et al.*, *ibid.*, **59**, 768 (1937); Marker and Rohrmann, *ibid.*, **61**, 3315 (1939).

contains one double bond more than estrone, has been established through the identity of its absorption spectrum with that of estrone,[467] through the formation of equilenin from equilin by palladium dehydrogenation,[468] and through its conversion to a glycol (XXXIII) containing
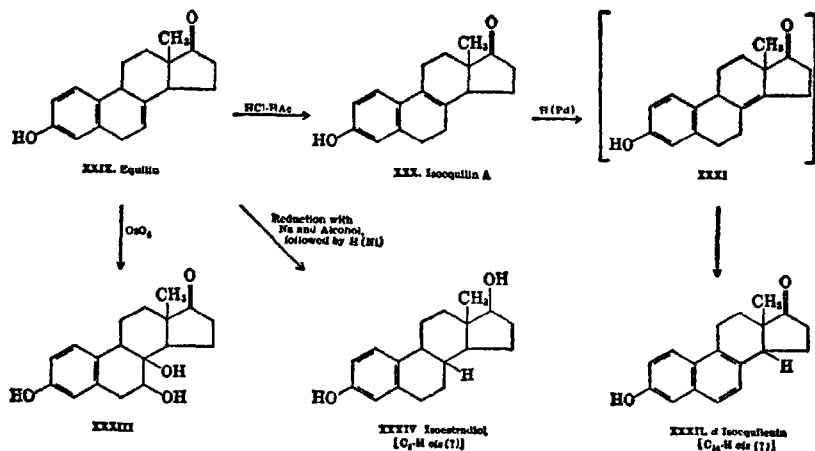
one secondary and one tertiary hydroxyl group by the action of osmium tetroxide.[469] Equilin is isomerized to isoequilin A (XXX) by boiling with hydrogen chloride-acetic acid.[470] On dehydrogenation, isoequilin A is converted to d-isoequilenin (XXXII), and, to account for the epimerization of the C_{14}—H, it is assumed to pass through the intermediate XXXI.[457] The glycol from equilin has been converted to 7-keto- and 7-hydroxy-estrones, but, in the course of their formation, epimerization of the C_8—H does not occur.[469] Epimerization of the C_8—H appears to be extremely probable in the case of isoestradiol, which is formed when 17-dihydroequilin [471] is treated with Raney nickel and hydrogen.[468] Here a disproportionation rather than uptake of hydrogen occurs, and 17(β)-dihydroequilenin and isoestradiol (cf. estradiol below) are formed. From isoestradiol a variety of isoestrane compounds have been prepared; the physiological potency of each is about one-third that of the corresponding estrane derivative. An isoequilin isomeric with isoequilin A has been prepared by dehydrobrominating dibromoandrostanedione (p. 1502); presumably the fourth double bond of isoequilin is situated at $C_5 : C_6$.[472]

[467] Cook and Roe, J. Soc. Chem. Ind., 54, 501 (1935).
[468] Direcherl and Hanusch, Z. physiol. Chem., 233, 13 (1935); 236, 131 (1935).
[469] Serini and Logemann, Ber., 71, 186 (1938); Pearlman and Wintersteiner, J. Biol. Chem., 130, 35 (1939).
[470] Hirschmann and Wintersteiner, J. Biol. Chem., 126, 737 (1938).
[471] David, Acta Brevia Neerland. Physiol. Pharmacol. Microbiol., 4, 63 (1934).
[472] Inhoffen, Naturwissenschaften, 25, 125 (1937).

Reduction Products of the Estrogens. When the 17-ketoestrogens are reduced in neutral or alkaline solution, only the carbonyl group is hydrogenated. With estrone two epimeric estradiols are obtained, especially if the reduction is conducted in alkaline solution.[473] By fractional crystallization from acetone and from alcohol, the mixture may be separated into α-estradiol (I), m.p. 176°, and β-estradiol (XXXV), m.p.223°; or, as Wintersteiner [473] has shown, the α-epimer may be isolated from the mixture by treatment in 80 per cent alcohol with digitonin with which it forms an insoluble precipitate. It is probable that the
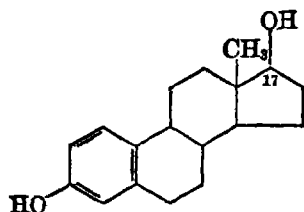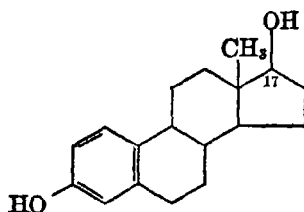
I. α-Estradiol   XXXV. β-Estradiol

C_{17}—OH of α-estradiol is *trans*, and that of β-estradiol *cis*, to the C_{13}—CH_3. The physical properties and the behavior towards dehydrating agents of these epimers furnish the evidence for these configurations. α-Estradiol has a lower melting point, a higher specific rotation, and a greater resistance to dehydrating agents than β-estradiol. By analogy to the epimeric testosterones (p. 1504), a higher melting point, a lower specific rotation, and lower resistance to dehydrating agents indicate a *cis* configuration of the C_{17}—OH and the C_{13}—CH_3 groups.

α-Estradiol has been isolated from the ovarian tissue of sows, from human placenta, and from horse testes;[474] it may be a true glandular sex hormone of other species, but this has not been demonstrated. Of the natural sex hormones, α-estradiol is the most potent estrogen known,* but its C_{17} epimer, β-estradiol, is even less active than estrone.

Catalytic reduction of the 17-ketoestrogens in acid media results first in hydrogenation of the carbonyl group to a 17(α)—OH, then in

[473] Schwenk and Hildebrandt, *Naturwissenschaften*, **21**, 177 (1933); Dirscherl, Z. *physiol. Chem.*, **239**, 53 (1936); Wintersteiner, *J. Am. Chem. Soc.*, **59**, 765 (1937); Whitman, Wintersteiner, and Schwenk, *J. Biol. Chem.*, **118**, 789 (1937); Butenandt and Goergens, *Z. physiol. Chem.*, **248**, 129 (1937); Marker and Rohrmann, *J. Am. Chem. Soc.*, **60**, 2927 (1938); U. S. pats.: Schwenk and Bradley, 2,072,830; Schoeller and Hildebrandt, 2,086,139; and Hildebrandt and Schwenk, 2,096,744.

[474] MacCorquodale, Thayer, and Doisy, *Proc. Soc., Exptl. Biol. Med.*, **32**, 1182 (1935); Westerfeld, MacCorquodale, Thayer, and Doisy, *J. Biol. Chem.*, **116**, 435 (1936); Huffmann, Thayer, and Doisy, *ibid.*, **133**, 567 (1940); Beall, *Biochem. J.*, **34**, 1293 (1940).

* An even more potent substance, $C_{19}H_{41}O_2N$, from ovarian tissue, was reported by Andrews and Fenger, *Endocrinology*, **20**, 563 (1936); but no further details have appeared.

hydrogenation of ring A, and finally in removal of the C_3—OH.[475] In the hydrogenation of ring A new centers of asymmetry are formed at C_8, C_5, and C_{10}; and thus eight hexahydro and sixteen octahydro derivatives are possible from estrone.

Norestrane Derivatives. As mentioned earlier, nearly all the attempts to synthesize the sex hormones have failed. This has been due largely to the difficulty of introducing both the angular methyl group at C_{13} and the carbonyl group at C_{17}. Among these synthetic efforts, the transformations leading to the x-norestrane* derivatives deserve special comment. Robinson was the first to synthesize x-norequilenin [476] and x-norestrane.[477] Starting with methoxynaphthyldiketoheptoic acid (XXXVI), prepared from furfurylidene-6-methoxy-2-acetylnaphthalene by boiling with alcoholic hydrochloric acid,[478] cyclization to XXXVII was effected by treatment with dilute aqueous potassium hydroxide. The methyl ester of this cyclized acid was hydrogenated, and then cyclized again by heating with sirupy phosphoric acid. The resulting diketonic derivative of methoxynorequilenin (XXXVIII, R = CH_3) lost the C_{11} carbonyl group on hydrogenation,† and from the reduction product a x-norequilenin was obtained by demethylation and oxidation.

Treatment of the acid XXXVII with acetic anhydride cyclized it to XXXIX (R = CH_3, R′ = Ac); and this product, after conversion to the dimethoxy derivative (XXXIX, R and R′ = CH_3), gave a cyanoketone (XL) by reacting the corresponding formylketone with hydroxylamine. Through alkaline hydrolysis, the cyanoketone was converted to the dicarboxylic acid XLI. This acid was hydrogenated in acetic acid with Adams catalyst to XLII. On pyrolysis of the lead salt of XLII, x-norestrone methyl ether (XLIII, R = CH_3) was obtained in good yield, and demethylation gave x-norestrone (XLIII, R = H). Apparently, neither the x-norequilenin nor the x-norestrone obtained by Robinson is physiologically active.

Goldberg and Müller [479] obtained x-norequilenin and active x-norestrone derivatives in another way. Starting with 1-ethinyl-6-methoxy-3,4-

[475] Dirscherl, *Z. physiol. Chem.*, **239**, 53 (1936); Marker, Kamm, Oakwood, and Tendick, *J. Am. Chem. Soc.*, **59**, 768 (1937); Ruzicka, Müller, and Mörgeli, *Helv. Chim. Acta*, **21**, 1394 (1938); Marker and Rohrmann, *J. Am. Chem. Soc.*, **60**, 2927 (1938); **61**, 3314 (1939); **62**, 73 (1940).

* x Indicates an unknown steric configuration.

[476] Robinson, *J. Chem. Soc.*, 1390 (1938); Koebner and Robinson, *ibid.*, 1994 (1938).

[477] Robinson and Rydon, *ibid.*, 1394 (1939).

[478] *Cf.* Kehrer and Igler, *Ber.*, **32**, 1178 (1899); **34**, 1263 (1903).

† A similar elimination of a carbonyl group by catalytic hydrogenation has been reported by Marker and Rohrmann, *J. Am. Chem. Soc.*, **61**, 3314 (1939). In this case the compound was probably 11-ketoequilenin, formed by chromic acid oxidation of equilenin.

[479] Goldberg and Müller, *Helv. Chim. Acta*, **23**, 831 (1940).

XXXVIII

CO₂H
CH₂—CH₂—CO₂H
XLI

XLIII

Reduction, and cyclisation with H₃PO₄

XXXVII

HO₂C
CH₂

CN
XL

KOH

aq. KOH

Pyrolysis of Pb salt

Ac₂O

Et formate and NaOET, followed by H₂NOH

XXXVI. Methoxynaphthyldiketo-heptoic acid (R = CH₃)

CO—CH₃
CH₃
CH₃
HO₂C

XXXIX

CO₂H
CH₂CH₂CO₂H
XLII

H (Pt)
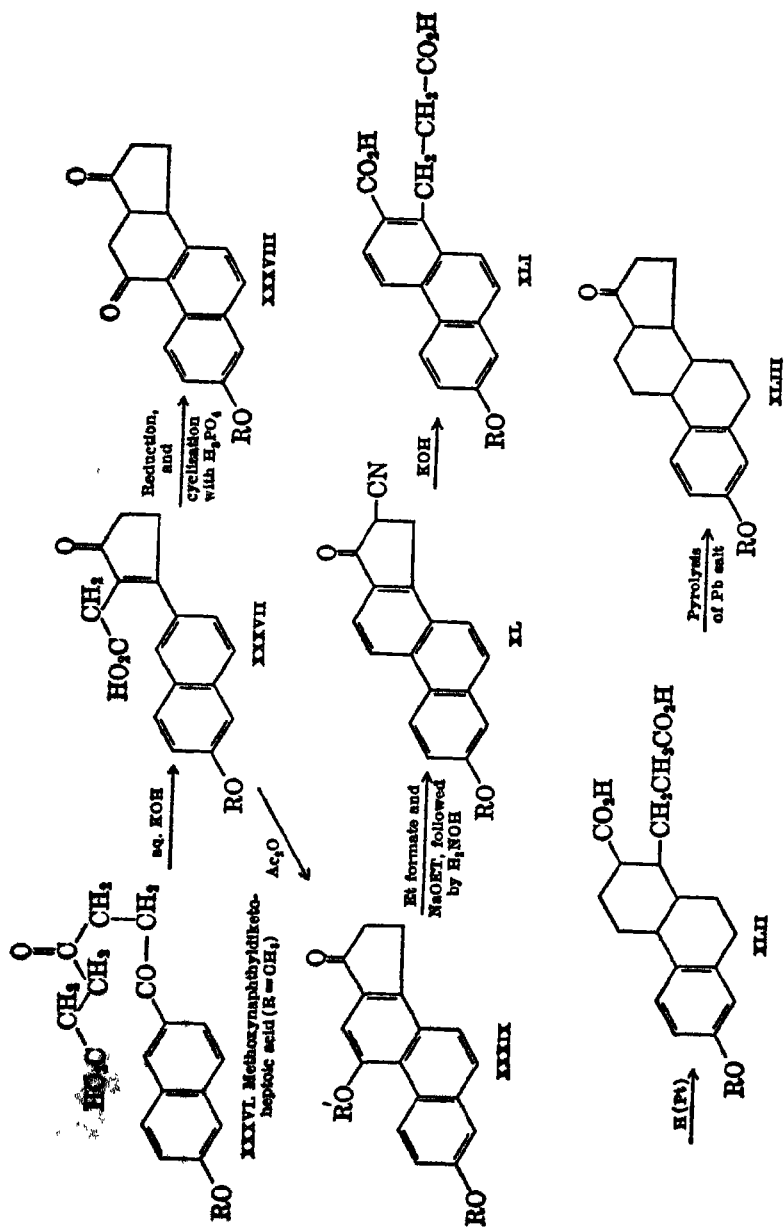
dihydronaphthalene (XLIV), partial hydrogenation gave the corresponding 1-vinyl derivative (XLV). By causing this product to react with α,β-diacetylethylene, two adducts, XLVI and XLVII, were obtained. Both these were converted to the same diacetyloctahydro-

XLIV. 1-Ethinyl-6-methoxy-
3,4-dihydronaphthalene

XLV. 1-Vinyl-6-methoxy-
3,4-dihydronaphthalene

XLVI

XLIX.
15-Methyl-15-dehydro-
x-norequilenin

XLIXa

and

XLVII

XLVIII

L

La
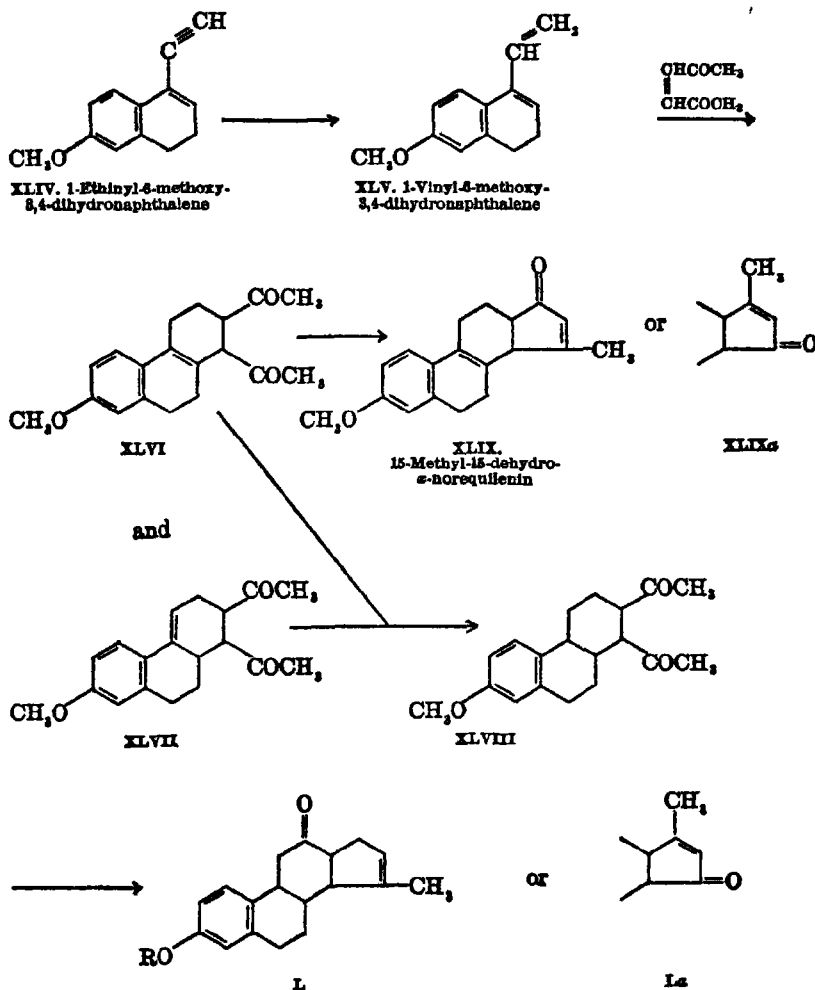
phenanthrene (XLVIII) by catalytic hydrogenation. On heating the higher-melting adduct with sodium methoxide in benzene, 15-methyl-15-dehydro-x-norequilenin methyl ether (XLIX or XLIXa) was obtained, and, on performing the same operation on XLVIII, 15-methyl-15-dehydro-x-norestrone methyl ether (L or La, R = CH₃) was formed.

Demethylation of the latter in the usual way gave the corresponding phenol (L or La, R = H). The product was a mixture of isomers, but in the physiological assay it was found to have an activity of 100μg./R. U.

Synthetic Estrogenic Compounds. A variety of synthetic products with estrogenic activity have been discovered.[480] Cook, Dodds, et al.,[481] prompted by the observation that 1-keto-1,2,3,4-tetrahydrophenanthrene (cf. XVI) produced estrus, examined a number of related compounds. Most of these proved to be inactive, or weakly active, but further search showed that certain 9,10-dialkyl derivatives of 9,10-dihydroxy-1,2,5,6-dibenz-9,10-dihydroanthracenes were potent, with the di-n-propyl derivative showing the maximum activity (0.5 mg. for estrus). Other alcohols such as 1,2-dihydroxy-1,2-di-(α-naphthyl)-acenaphthene and diphenyl α-naphthyl carbinol were found to be weakly active,[482] but it was not until phenolic substances were studied that high estrogenic potency was obtained. The phenols examined were the mono- and dihydroxy derivatives of diphenyl,[483] and of various alkyl and aryl nuclear-substituted diphenyl methanes and stilbenes.[484] One of the most active of these was 4,4'-dihydroxystilbene (0.5 mg. for estrus), which was named stilbestrol.* The C-alkylated stilbestrols proved to be far more potent than the parent compound, and α,β-diethylstilbestrol (LI) was found to be equal in potency (0.3 μg. for estrus) to the natural estrogens.[485] Since diethylstilbestrol can exist in two steric modifications (cis and trans), attempts have been made to isolate the two forms. These have failed, however, and the assumption that a trans configuration is present in the active preparation seems to be unproved on the basis of the present evidence.†

[480] v. Wessely, Angew. Chem., **53**, 197 (1940), lists about sixty compounds.

[481] Cook, Dodds, Hewett, and Lawson, Proc. Roy. Soc. (London), **114B**, 272 (1934); Cook, Dodds, and Greenwood, ibid., **114B**, 286 (1934).

[482] Dodds, Helv. Chim. Acta, **19**, E49 (1936); Dodds and Lawson, Nature, **139**, 627 (1937).

[483] Dodds and Lawson, Proc. Roy. Soc. (London), **125B**, 222 (1938).

[484] Dodds and Lawson, Nature, **139**, 627, 1068 (1937); Dodds, Fitzgerald, and Lawson, ibid., **140**, 772 (1937).

* In the course of the early work a very potent substance was occasionally obtained by alkaline demethylation of anethole (4-propenylanisol), a natural ether. This very potent product has been identified as dihydrodiethylstilbestrol by Campbell, Dodds, and Lawson, Proc. Roy. Soc. (London), **125B**, 253 (1940).

[485] Dodds, Goldberg, Lawson, and Robinson, Nature, **141**, 247 (1938); **142**, 32, 211 (1938); Proc. Roy. Soc. (London), **127B**, 140 (1939).

† The work of v. Wessely and Welleba, Ber., **74**, 777, 785 (1941), presents evidence for trans configuration of the known diethylstilbestrol. In particular, hydrogenation of diethylstilbestrol or of its dimethyl ether gives 88 and 97 per cent, respectively, of the corresponding racemic dihydro products. Under the same conditions, the trans form of α,β-dimethylstilbene is converted in 98 per cent yield to the racemic α,β-dimethyldihydrostilbene, and the cis form in 99 per cent yield to meso-α,β-dimethyldihydrostilbene.

$$\text{HO}-\langle\bigcirc\rangle-\underset{\underset{C_2H_5}{|}}{C}=\underset{\underset{C_2H_5}{|}}{C}-\langle\bigcirc\rangle-\text{OH}$$

LI.   Diethylstilbestrol

$$\text{HO}-\langle\bigcirc\rangle-\underset{\underset{\text{CH}_3-\text{CH}}{\|}}{C}-\underset{\underset{\text{H}}{|}}{C}-\langle\bigcirc\rangle-\text{OH}$$

LII.   Di-(4-hydroxyphenyl)-hexene

$$\underset{\text{LIII. Desoxyanisoin}}{\text{MeOC}_6\text{H}_4-\overset{\overset{O}{\|}}{C}-\text{CH}_2-\text{C}_6\text{H}_4\text{OMe}} \xrightarrow[\text{(NaOEt)}]{\text{RX}} \underset{\text{LIV}}{\text{MeOC}_6\text{H}_4-\overset{\overset{O}{\|}}{C}-\overset{\overset{R}{|}}{C}\text{H}-\text{C}_6\text{H}_4\text{OMe}} \xrightarrow{\text{R'MgX}}$$

$$\underset{\text{LV}}{\text{MeOC}_6\text{H}_4-\overset{\overset{R'}{|}}{\underset{\underset{\text{OH}}{|}}{C}}-\overset{\overset{R}{|}}{\underset{\underset{\text{H}}{|}}{C}}-\text{C}_6\text{H}_4\text{OMe}} \xrightarrow[\substack{\text{and} \\ \text{demethylation}}]{\text{Dehydration}} \underset{\text{LVI}}{\text{HOC}_6\text{H}_4-\overset{\overset{R'}{|}}{C}=\overset{\overset{R}{|}}{C}-\text{C}_6\text{H}_4\text{OH}}$$
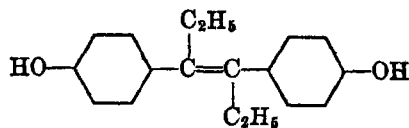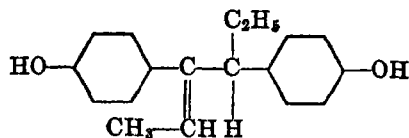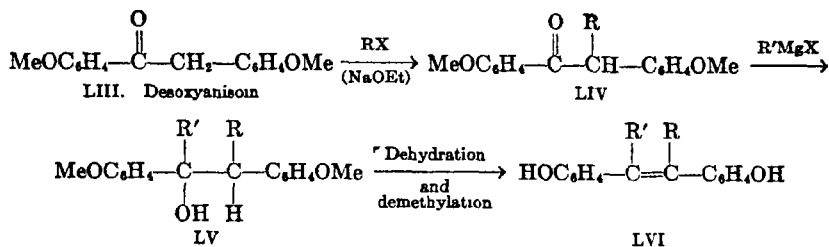
That steric configuration plays a part in the activity is clearly brought out in the two isomeric di-(4-hydroxyphenyl)-hexenes of structure LII.[486] The higher-melting isomer, m.p. 153°, has approximately one-tenth the potency of diethylstilbestrol, while the lower-melting isomer, m.p. 143°, has an activity of one-hundredth that of diethylstilbestrol. On treatment with iodine, both forms of the hexene are converted to diethylstilbestrol. A double bond in the aliphatic portion of the stilbestrol molecule is not essential to high activity. This is evident from the fact that dihydrodiethylstilbestrol, or hexestrol (0.2 μg. for estrus), is as active as diethylstilbestrol itself.[487]

Practically, the discovery of the dialkylstilbestrols is extremely important since this group of compounds may be synthesized readily from available materials. The following general procedure has been used for the preparation of the C-alkylstilbesterols. Desoxyanisoin (LIII), on treatment with an alkyl halide in the presence of sodium ethoxide, is substituted adjacent to the carbonyl group and gives a ketone (LIV), which readily reacts with Grignard reagents to form carbinols (LV). On dehydration followed by demethylation, the dialkylstilbesterols (LVI) are obtained.*

[486] v. Wessely and Kleedorfer, *Naturwissenschaften*, **27**, 567 (1939).

[487] Campbell, Dodds, and Lawson, *Nature*, **142**, 1121 (1938); Kreschbaum, Kleedorfer, Prillinger, v. Wessely, and Zajic, *Naturwissenschaften*, **27**, 131 (1939); Docken and Spielman, *J. Am. Chem. Soc.*, **62**, 2163 (1940); Bernstein and Wallis, *ibid.*, **62**, 2871 (1940).

* The preparation of a number of synthetic estrogens *via* the route ketazine → azine tetrahydride → azine dihydride → diphenylethane has been described by Bretschneider *et al.*, *Ber.*, **74**, 571 (1941), and by Földi and Fodor, *Ber.*, **74**, 589 (1941).

Physiological Relationships of the Estrogens. In the estrane group physiological activity is primarily dependent on the presence of an hydroxyl group at C_3. For example, Bachmann [488] has shown that synthetic 17-equilenone and 6-hydroxy-17-equilenone are inactive. Where an hydroxyl group is present at C_3, the degree of activity is dependent upon the steric configurations of rings B/C and C/D, and on the steric position of the hydroxyl group at C_{17}. A *trans* configuration of rings B/C and C/D is essential for maximum activity. This is shown by the relative potencies of equilin and isoequilin, or of the equilenins and the isoequilenins. The large difference between the potency of estrone and that of equilenin further illustrates the importance of the configuration of rings B/C. Similarly, the difference in potency of α-estradiol and of β-estradiol shows the effect of the steric position of the C_{17} hydroxyl group. In making these generalizations, it may be noted that the potencies assigned to the several estrogens in Table X, and elsewhere, probably do not express the ability of each compound to supplement deficiencies due to glandular disfunction.

In clinical use the natural and artificial estrogens have been widely employed. To supplement or to replace the natural glandular secretion of hormones, various derivatives of the estrogens are injected or administered orally. Because of its prolonged effect, the C_3 benzoate of α-estradiol has been widely employed for intramuscular injections.[489] Other esters have been described,[490] but none seems as satisfactory as the C_3 benzoate. For oral use, estriol glucuronide and the 17-ethinyl derivative of estradiol, formed by the action of potassium acetylide on estrone in liquid ammonia,[491] seem to be the most satisfactory of the derivatives which have been studied.* Diethylstilbestrol and related compounds are especially suitable for oral therapy, but their clinical use has been attended by a number of undesirable secondary effects.[492]

[488] Bachmann and Wilds, *J. Am. Chem. Soc.*, **62**, 2084 (1940); Bachmann and Holmes, *ibid.*, **62**, 2750 (1940).

[489] David, de Jongh, and Laqueur, *Arch. intern. pharmacodynamie*, **51**, 137 (1937).

[490] Dirscherl, *Z. physiol. Chem.*, **239**, 49 (1936); Miescher and Scholz, *Helv. Chim. Acta*, **20**, 1237 (1937); Miescher, Scholz, and Tschopp, *Biochem. J.*, **32**, 1273 (1938); Marker and Rohrmann, *J. Am. Chem. Soc.*, **61**, 1927, 2974 (1939); U. S. pats.: Ach and Dirscherl, 2,031,581; Schwenk and Hildebrandt, 2,033,487; Hildebrandt and Schwenk, 2,154,272; Miescher and Scholz, 2,156,599, and 2,160,555; and Weisz, 2,167,132.

[491] Inhoffen, Logemann, Hohlweg, and Serini, *Ber.*, **71**, 1024 (1938).

* From the tuberous growth at the end of the roots of the rare creeping vine, *Butea superba*, indigenous to the northern part of Thailand, a substance called tokokinin, $C_{18}H_{28}O_5(?)$, m.p. 260°, has been isolated. This compound is twice as active as estrone when given subcutaneously, and one hundred times as active when given orally. Preliminary investigations of the physiological effects have been reported by Schoeller, Dohrn, and Hohlweg, *Naturwissenschaften*, **28**, 532 (1940), and of the chemical study by Butenandt, *ibid.*, **28**, 533 (1940).

[492] Kennaway et al., Atkinson, *Endocrinology*, **27**, 581 (1940); v. Wessely, *Angew Chem.*, **53**, 197 (1940); Shorr, Robinson, and Papanicolaou, *J. Am. Med. Assoc.*, **113**, 2312 (1939).

The excretion of the estrogens in the urine is usually in the form of oxidized rather than hydrogenated compounds. As mentioned earlier, some of these are excreted as glucuronides and some as sulfates. In addition, small amounts of two isomeric forms of estranediol have been isolated from normal female urine, and $\Delta^{5,7,9}$-estratrien-3(β?)-ol-20-one from mares' pregnancy urine.[493] The isolation of these compounds probably indicates that the body is capable of hydrogenating ring A of the estrogens.

Progesterone and Pregnane Derivatives

In the sexual cycle of the female the estrogenic hormones bring the animal into heat and, at the same time, an ovum begins to mature. At the time when the ovum descends into the uterine passages, the corpus luteum, a small yellow body in the ovary, begins to produce the hormone progesterone. Just as the estrogenic hormones bring about a condition favorable to mating in the female, so does the corpus luteum hormone by causing a proliferation of the endometrium (the lining of the uterus) create a condition ideal for the implantation of the fertilized ovum. If implantation occurs, the corpus luteum persists and continues to furnish its hormone during most of the remainder of pregnancy in the majority of species. In the absence of implantation the corpus luteum degenerates and the animal once more comes into estrus or lapses into anestrus, depending on the nature of her cycle. So, through the complementary action of the estrogenic hormones and of the corpus luteum hormone, the sexual cycle in females is controlled.

The isolation of progesterone * was made possible by Corner and W. M. Allen [494] through the development of a biological test to measure its activity. In this test a female rabbit is castrated a day after mating and an oil solution of the hormone or hormonal preparation is injected subcutaneously each day for five days. A total of 1 mg. (1 rabbit unit) of pure progesterone brings the endometrium into complete proliferation, which is easily recognized in a histological section from the uterus of the animal on the sixth day. Clauberg [495] has developed a modification of this test by using immature rabbits and preparing them for the assay by maturing the uterus through injection of estrogenic hormones. Progesterone has no effect on the uterus of immature rabbits.

. [493] Marker, Rohrmann, Lawson, and Wittle, *J. Am. Chem. Soc.*, **60**, 1901 (1938); Heard and McKay, *J. Biol. Chem.*, **135**, 801 (1940).

* The name progesterone was adopted by international agreement [Allen, Butenandt, Corner, and Slotta, *Science*, **82**, 153 (1935); *Ber.*, **68**, 1746 (1935); *Nature*, **136**, 303 (1935)]. Prior to this it was known as progestin, the corpus luteum hormone, or luteosterone.

[494] Corner and Allen, *Am. J. Physiol.*, **86**, 74 (1928); **88**, 326 (1929); Allen and Corner, *ibid.*, **88**, 340 (1929).

[495] See Clauberg, "Die weibliche Sexualhormone," Springer, Berlin (1933); Butenandt, Westphal, and Hohlweg, *Z. physiol. Chem.*, **227**, 84 (1934).

Isolation of Progesterone. Following the development of the Allen-Corner test, Allen [496] isolated potent fractions from the corpus luteum of pregnant sows, but the isolation of the pure hormone was first announced by Butenandt.[497] Shortly after this Slotta et al.,[498] Allen and Wintersteiner,[499] and Hartmann and Wettstein [500] also described pure preparations. The hormone occurs in two crystalline modifications: α-progesterone, m.p. 128.5° (prisms), and β-progesterone, m.p. 121° (needles), both of the same physiological potency. By refined procedures for the isolation of progesterone, 20–30 mg. of both forms of the hormone may be isolated from 250 kg. of sows' ovaries.[501] Accompanying progesterone in the extracts is a small amount of *allo*pregnan-3 (β)-ol-20-one (*cf.* formula LX), which is probably a biochemical reduction product of the hormone.[502]

The Structure of Progesterone. The hormone progesterone, $C_{21}H_{30}O_2$, is a diketone with strong absorption at 240 mμ (α,β-unsaturated ketone). The structure of the hormone was first suggested by Slotta,[503] but the proof of this constitution is due largely to Butenandt.[504] In the work which established the structure, stigmasterol (p. 1396) was converted through the acetate of 3(β)-hydroxy-Δ⁵-bisnorcholenic acid (LVII) to Δ⁵-pregnen-3(β)-ol-20-one (LVIII). Originally, the latter was oxidized and rearranged to progesterone (II) by direct oxidation with chromic acid [504] or with copper oxide.[505] Subsequently, the conversion

LVII 3-(β) Hydroxy-Δ⁵-bisnorcholenic acid — LVIII. Δ⁵-Pregnen-3(β)-ol-20-one — II. Progesterone (Δ⁴-pregnene-3,20-dione)
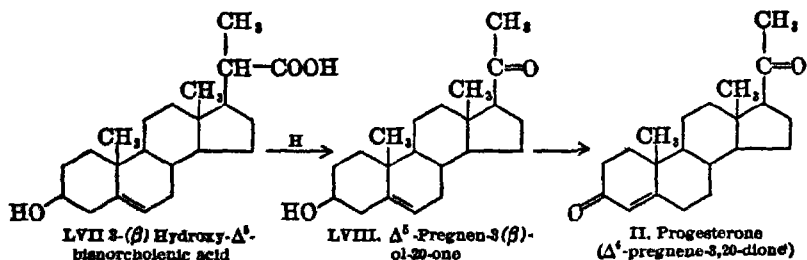
[496] Allen, *J. Biol. Chem.*, **98**, 591 (1932).

[497] Butenandt, *Verhandl. deut. Ges. inn. Med.* (April, 1934); Butenandt and Westphal, *Ber.*, **67**, 1440 (1934); Butenandt, Westphal, and Hohlweg. *Z. physiol. Chem.*, **227**, 84 (1934).

[498] Slotta, Ruschig, and Fels, *Ber.*, **67**, 1624 (1934); *cf.* Neuhaus, *Ber.*, **67**, 1627 (1934).

[499] Allen and Wintersteiner, *Science*, **80**, 190 (1934); Wintersteiner and Allen, *J. Biol. Chem.*, **107**, 321 (1934).

[500] Hartmann and Wettstein, *Helv. Chim. Acta*, **17**, 878, 1365 (1934); U. S. pats.: 2,062,9.. (reissue 21,064) and 2,092,453.

[501] Allen and Goetsch, *J. Biol. Chem.*, **116**, 635 (1936); Butenandt and Westphal, *Ber.*, ..3 (1936).

[502] Butenandt and Mamoli, *Ber.*, **67**, 1897 (1934); Fernholz, *Z. physiol. Chem.*, **230**, 155 (1934).

[503] Slotta, Ruschig, and Fels, *Klin. Wochschr.*, **13**, 1207 (1934).

[504] Butenandt, Westphal, and Cobler, *Ber.*, **67**, 1611 (1934).

[505] Butenandt et. al., *Ber.*, **67**, 1901, 2085 (1934).

has been carried out by bromination, followed by oxidation and debromination,[506] and by dehydrogenation and rearrangement with Oppenauer's reagent (aluminum alkoxides)[507] or with metals such as platinum black.[508] Progesterone may be prepared also by brominating 3,20-pregnanedione (LXIV) at C_4 and removing hydrogen and bromine by treatment with pyridine.[509],*

Pregnane and *Allo*pregnane Derivatives. Progesterone is but one of a group of saturated and unsaturated steroids derived from the hydrocarbons, pregnane (LIX), m.p. 83.5°,[510] and *allo*pregnane (LX), m.p. 84°.[511] Some of the naturally occurring members of this group are bio-

LIX. Pregnane   LX. *Allo*pregnane
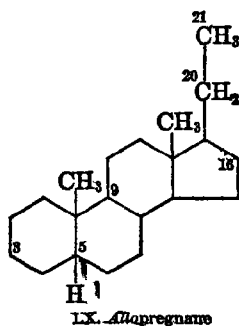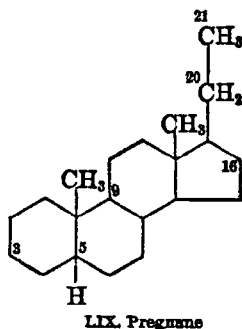
logical reduction products of progesterone and usually are found in pregnancy urine. In addition, many of the adrenal substances (p. 1510) are also derivatives of this group. The preparation of the pregnanes from other steroids usually is involved. From the pseudosapogenins (p. 1462), however, Marker [512] has shown that the side chain may be removed by oxidation to give excellent yields of Δ^{16}-pregnenolones, and, from these, various substituted pregnanes and *allo*pregnanes may be obtained. In Table XI the more important members of this group are listed together with their sources.

[506] Fernholz, *Ber.*, **67**, 1855, 2027 (1934).

[507] Oppenauer, *Rec. trav. chim.*, **56**, 137 (1937).

[508] Marker and Krueger, *J. Am. Chem. Soc.*, **62**, 3349 (1940).

[509] Butenandt and Schmidt, *Ber.*, **67**, 1901, 2088 (1934); Serini, Strassberger, and Butenandt, U. S. pat., 2,153,700.

* *Cf.* p. 1506 for other methods of preparing progesterone. The conversion of cholesterol to progesterone by direct oxidation has been described by Tavastsherna, *Arch. Sci. Biol. (U.S.S.R.)*, **40**, 141 (1936) [*C. A.*, **31**, 6670 (1937)], and by Spielman and Meyer, *J. Am. Chem. Soc.*, **61**, 893 (1939). The latter authors report a 0.3 per cent yield of crude progesterone. The hormone has been obtained also by Dirscherl and Hanusch, *Z. physiol. Chem.*, **257**, 49 (1939), from cholestenone dibromide or from cholestenone.

[510] Butenandt, Hildebrandt, and Brücher, *Ber.*, **64**, 2529 (1931).

[511] Steiger and Reichstein, *Nature*, **141**, 208 (1938); Marker *et al.*, *J. Am. Chem. Soc* **60**, 1061 (1938).

[512] *Inter al.*, Marker, *J. Am. Chem. Soc.*, **62**, 3350 (1940).

TABLE XI

Pregnane and *Allo*pregnane Derivatives *

Compound	M.P. ° C.	Source or Derivation
Unsaturated Dialcohols, $C_{21}H_{34}O_2$		
Δ^5-Pregnene-3(β),20(α)-diol	176	Mares' pregnancy urine.
Dialcohols, $C_{21}H_{36}O_2$		
3(α),20(α)-Pregnanediol	236	Pregnancy urine of women, cows, and mares; urine of women during secretion phase of menstrual cycle; bulls' urine; Na-EtOH reduction of Δ^{16}-pregnene-3,20-dione.
3(α),20(β)-Pregnanediol	231–234	H(Pt) reduction of pregnan-3(α)-ol-20-one in neutral or acid media.
3(β),20(α)-Pregnanediol	182	H(Pt) reduction of pregnan-20(α)-ol-3-one in acid media.
3(β),20(β)-Pregnanediol	174–176	H(Pt) reduction of 3,20-pregnanedione in acid media.
3(α),20(α)-*Allo*pregnanediol	248	Pregnancy urine of women, cows, and mares; urine of women, stallions, and bulls.
3(α),20(β)-*Allo*pregnanediol	207	H(Pt) reduction of *allo*pregnan-3(α)-ol-20-one in acid media.
3(β),20(α)-*Allo*pregnanediol	215	Na-EtOH reduction of Δ^{16}-*allo*pregnene-3,20-dione
3(β),20(β)-*Allo*pregnanediol	192–194	H(Pt) reduction of 3,20-*allo*pregnanedione in neutral media.
Ketonic Alcohols, $C_{21}H_{34}O_2$		
Pregnan-3(α)-ol-20-one	144	Pregnancy urine of women and sows; partial hydrogenation in neutral media of 3,20-pregnanedione.
Pregnan-3(β)-ol-20-one	149	Partial hydrogenation (Pt) in acid media of 3,20-pregnanedione.
Pregnan-20(α)-ol-3-one	152	Oxidation of C_{20} esters of 3(α),20(α)-pregnanediol.
Pregnan-20(β)-ol-3-one	172	Oxidation of C_{20} esters of 3(β),20(β)-pregnanediol.
*Allo*pregnan-3(α)-ol-20-one	176	Pregnancy urine of women; oxidation of C_2 esters of 3(α),20(α)-*allo*pregnanediol.
*Allo*pregnan-3(β)-ol-20-one	194	Corpus luteum of sows and cows; adrenals of cows; mares' pregnancy urine; sows' urine; degradation of stigmasterol
*Allo*pregnan-20(α)-ol-3-one	128	Oxidation of C_{20} esters of 3(α),20(α)-*allo*pregnanediol.
*Allo*pregnan-20(β)-ol-3-one	195	Oxidation of C_{20} esters of 3(β),20(β)-*allo*pregnanediol.
Diketones, $C_{21}H_{32}O_2$		
3,20-Pregnanedione	118	Pregnancy urine of mares; CrO_3 oxidation of 3(α),20(α)-pregnanediol.
3,20-*Allo*pregnanedione	200	Pregnancy urine of mares; CrO_3 oxidation of 3(α),20(α)-*allo*pregnanediol.

* Data from Marker et al., *J. Am. Chem. Soc.*, **59**, 2291 (1937); **61**, 588 (1939); **62**, 518, 898 (1940); and from Westphal, *Naturwissenschaften*, **28**, 465 (1940).

Of the urinary pregnanes, 3(α),20(α)-pregnanediol (LXIII) is present (as the glucuronide) during pregnancy in the largest quantity, and was the first to be isolated.[513] Its nuclear structure has been determined by the conversion of the diol to pregnane via 3,20-pregnanedione,[514] and the hydroxyl groups have been allocated most simply by the conversion of the latter to progesterone. The preparation of pregnane from etiocholyl methyl ketone by Clemmensen reduction has established the spatial configuration of the nucleus.[514] Early in the study of the diol, an α-configuration of the C_3—OH group was indicated by the failure of 3(α),20 (α)-pregnanediol to form an insoluble digitonide, and this has been confirmed by chemical transformations. The steric position of the C_{20}—OH group cannot be determined unequivocally, but Marker [515] has suggested that this be regarded arbitrarily as having an α-configuration. Study of the four theoretically possible epimers of pregnanediol has shown that this convention is satisfactory.

The natural 3(α),20(α)-pregnanediol cannot be prepared by hydrogenation of 3,20-pregnanedione (LXIV) but has been obtained only by reduction of Δ^{16}-pregnen-3(α)-ol-20-one acetate (LXIIa) with sodium and alcohol.[516] The enolone has been prepared from pseudoepisarsasapogenin acetate (LXI) by chromic acid oxidation at room temperature. When pseudosarsasapogenin acetate, rather than the pseudoepisapogenin, is oxidized with chromic acid, Δ^{16}-pregnen-3(β)-ol-20-one results, and from this 3(β),20(α)-pregnanediol is obtained on reduction with sodium and alcohol.[516] Catalytic hydrogenation of 3,20-pregnanedione (LXIV) with platinum as the catalyst leads to the formation of 3(α),20(β)-pregnanediol (LXVI) in acid media, and to the production of 3(β),20(β)-pregnanediol (LXVIII) in neutral media.[515] In both cases the 3-carbonyl group is hydrogenated first, and the intermediates, pregnan-3(α)-ol-20-one (LXV) and pregnan-3(β)-ol-20-one (LXVII), are readily obtained by partial hydrogenation. The Δ^{16}-pregnenolones are reduced in the nucleus if a palladium-barium sulfate catalyst is employed, and are completely hydrogenated with the formation of a β-configuration of the C_{20}—OH group if platinum is used. Thus, Δ^{16}-pregnen-3(α)-ol-20-one (LXIIb) with palladium-barium sulfate catalyst is hydrogenated to pregnan-3(α)-ol-20-one, and with platinum catalyst is reduced to 3(α),20(β)-pregnanediol (LXVI).[512] By analogous procedures the epimeric allopregnanediols and allopregnanolones have been

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[513] Marrian, Biochem. J., 23, 1090 (1929); Butenandt, Ber., 63, 659 (1930); Dingemanse et al., Deut. med. Wochschr., 56, 301 (1930).

[514] Butenandt, Hildebrandt, and Brücher, Ber., 64, 2529 (1931).

[515] Marker et. al., J. Am. Chem. Soc., 59, 2291 (1937); Marker and Lawson, ibid., 61, 588 (1939).

[516] Marker and Rohrmann, ibid., 62, 518 (1940).

LXIII. 3(α),20(α)-Pregnanediol

LXVI. 3(α),20(β)-Pregnanediol

LXVIII. 3(β),20(β)-Pregnanediol

Na and ethanol

H (Pt)

LXII. Δ¹⁶-Pregnen-3(α)-ol-20-one
LXIIa. R = Ac
LXIIb. R = H

H (Pd-BaSO₄)

LXV. Pregnan-3(α)-ol-20-one

LXVII. Pregnan-6(β)-ol-20-one

CrO₃

LXI. Pseudoplasmasapogenin acetate (provisional)

Acid media

H (Pt)

Neutral media

LXIV. 3,20-Pregnanedione
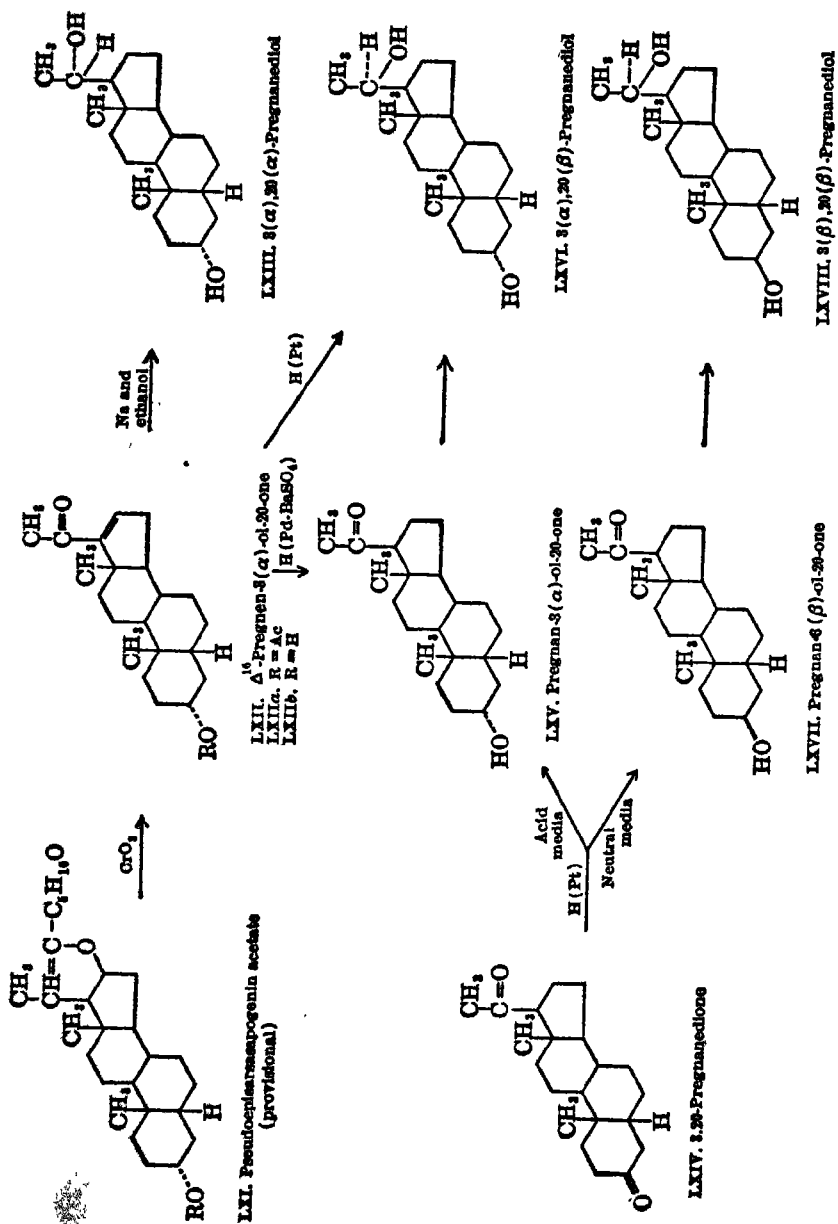
prepared from 3,20-*allo*pregnanedione and from the Δ^{16}-*allo*pregnen-olones.[515, 517]

From these results, largely due to Marker and co-workers, it is evident that catalytic reduction of the ketonic pregnanes in different media produces a β-configuration of the C_{20}—OH group, and that only by alkaline reduction with sodium and alcohol can an α-configuration be obtained. Generally, in the reduction of the C_3 carbonyl groups, iso-meric mixtures are formed, with acid media leading to a predominance of the compound with a *cis* relationship between the C_3—OH and the C_5—H, and with neutral media giving a larger proportion of the com-pound with a *trans* relationship of these two centers of asymmetry. Thus, von Auwers-Skita's rule (p. 1373) holds qualitatively. By heating with sodium and xylene, the C_3—OH groups can be epimerized in the normal way so that the compound with a *trans* relationship of the C_3—OH to the C_5—H predominates, but the C_{20}—OH groups cannot be epimerized in this way. The C_3—OH groups, whether in α- or β-configuration, are always more reactive than C_{20}—OH groups in either configuration. Because of this difference in reactivity, it is possible with the pregnanediols to acetylate preferentially a C_3—OH group or to deacetylate selectively a C_3 acetate without affecting a C_{20} acetate.

In addition to the urinary pregnane derivatives given in Table XI, $3(\alpha),16,20$-*allo*pregnanetriol (LXIX) has been isolated from pregnancy urine.[518] When the triol is heated with aluminum isopropoxide, oxida-tion and dehydration take place, and Δ^{16}-*allo*pregnene-3,20-dione (LXX) is obtained.[519] The same product is formed by oxidation of pseudotigogenin with chromic acid under mild conditions. The dehy-dration, with involvement of the C_{16}—OH group in these two transfor-mations, apparently is related to the steric configuration of rings A/B. In contrast, $3(\beta),16,20$-pregnanetriol (LXXI), formed by oxidation of pseudosarsasapogenin, is oxidized and dehydrated by aluminum iso-propoxide to $\Delta^{17(20)}$-pregnene-3,16-dione (LXXII).

When *allo*pregnan-3(β)-ol-20-one, m.p. 194.5°, $[\alpha]_D + 90.8°$, is re-fluxed in 5 per cent methanolic alkali, a rearrangement occurs, and an iso*allo*pregnanolone, m.p. 147–148°, $[\alpha]_D + 6°$, is formed in about 30 per cent yield.[520] The change is reversed by the addition of acid, and is easily followed by measuring the optical rotation of the solution. The conversion has been demonstrated also for *allo*pregnanolone → iso-*allo*pregnanolone, 3,20-pregnanedione → 3,20-isopregnanedione, and Δ^5-

[517] Hartmann and Locher, *Helv. Chim. Acta*, **18**, 160 (1935); Marker and Rohrmann, *J. Am. Chem. Soc.*, **62**, 898 (1940).

[518] Haslewood, Marrian, and Smith, *Biochem. J.*, **28**, 1316 (1934); Odell and Marrian, *J. Biol. Chem.*, **128**, 333 (1938); Marker and Wittle, *J. Am. Chem. Soc.*, **61**, 855 (1939).

[519] Marker and Turner, *ibid.*, **62**, 2540 (1940).

[520] Butenandt and Mamoli, *Ber.*, **68**, 1847 (1935).

pregnenolone → Δ⁵ isopregnenedione; the change isoprogesterone → progesterone has been realized, but not the reverse.[521] The isomerization apparently involves only the rearrangement of the C_{17}—$COCH_3$ group,

CH₃
|
CHOH
        CH₃   OH

CH₃

HO   H

LXIX. 3 (α),16,20-Allopregnanetriol

CH₃
|
CHOH
        CH₃

CH₃

O   H

LXX. Δ¹⁶-Allopregnane-3,20-dione

CH₃
|
CHOH
        CH₃   OH

CH₃

HO   H

LXXI. 3 (β),16,20-Pregnanetriol

CH₃
|
CH
        CH₃   O

CH₃

O   H

LXXII. Δ¹⁷(²⁰)-Pregnene-3,16-dione (provisional)

CH₃
|
C=O
CH₃

Persulfuric acid (Caro's reagent)
→

LXXIII. 20-Pregnanones

CH₃
|
C=O
|
O
CH₃

LXXIV. Androstan-17(α)-ol acetates

+

CH₂OAc
|
C=O
CH₃

LXXV. Pregnan-21-ol-20-one acetates

+

CO₂H
|
CH₃

LXXVI. Etiocholanic acids

but the iso compounds with a β-configuration of the C_3—OH group do not form insoluble digitonides.

When stirred at 25° in acetic acid solution with persulfuric acid

⁵²¹ Butenandt and Fleischer, *Ber.*, **70**, 96 (1937); Butenandt, Schmidt-Thomé, and Weiss, *Ber.*, **72**, 1112 (1939).

(Caro's reagent), the 20-pregnanones (LXXIII) are converted to a mixture of androstan-17(α)-ol acetates (LXXIV) and pregnan-21-ol-20-one acetates (LXXV).[522] Both are obtained in about 30 per cent yield. The reaction affords a direct transition from the pregnanes to the androstanes (p. 1499) and, by oxidation of the pregnan-21-ol-20-ones, to the etiocholanic acids (LXXVI). The mechanism of the transformation is obscure, but Baeyer and Villiger,[523] who discovered the effect of Caro's reagent on ketones, have suggested that a polymerized peroxide is an intermediate product, and that the rearrangement of the peroxide may be analogous to the Beckmann rearrangement of oximes.

The Pregnenes. The methods of introducing unsaturation in the pregnanes are essentially the same as those discussed in connection with the other steroids. Thus, from 2-bromo*allo*pregnane-3,20-dione, Δ¹-*allo*pregnane-3,20-dione is obtained by heating with pyridine bases, and *h*-Δ¹-*allo*pregnene-3,20-dione by reacting with potassium acetate.[118] As mentioned earlier, Δ⁴-pregnene-3,20-dione (progesterone) is formed when 4-bromopregnane-3,20-dione is treated with pyridine. Because of the importance of progesterone, a number of ingenious methods have been developed for its production or for the production of the intermediate Δ⁵-pregnen-3(β)-ol-20-one. Although this intermediate may be isolated from the mixture obtained by the oxidation of 5,6-dibromo-sterols, the yield is low and the isolation involved.[524] More satisfactory is the degradation of 3(β)-hydroxy-Δ⁵-bisnorcholenic acid by Barbier-Wieland's method (p. 1357) or by Curtius degradation *via* the azide through the C_{20} amine and the C_{20} alcohol.[525] Preferential hydrogenation with palladium-barium sulfate of a C_{16} ethylenic linkage in Δ⁵,¹⁶-pregnadien-3(β)-ol-20-one, formed by oxidation of pseudodiosgenin derivatives, leads also to Δ⁵-pregnen-3(β)-ol-20-one.[526] The most satisfactory method of dehydrogenating and rearranging pregnenolone to progesterone is by the use of Oppenauer's method. If quinone is used in place of acetone or cyclohexanone as the hydrogen acceptor, dehydrogenation at C_6 takes place in addition to the normal change and 6-dehydroprogesterone is formed.[527] Other transformations leading to the pregnenes are discussed in connection with the members of the androstane group and of the adrenal substances.

[522] Marker *et al.*, *J. Am. Chem. Soc.*, **62**, 650, 2543 (1940).

[523] Baeyer and Villiger, *Ber.*, **32**, 3625 (1899); **33**, 858 (1900); *cf.* Rollett and Bratke, *Monatsch.*, **43**, 685 (1922); Rusicka and Stoll, *Helv. Chim. Acta*, **11**, 1159 (1928).

[524] Fujii and Matsukawa, *J. Pharm. Soc. Japan*, **56**, 24 (1936) [*Chem. Zentr.*, (II) 1354 (1936)]; Rusicka and Fischer, *Helv. Chim. Acta*, **20**, 1291 (1937).

[525] Ehrhart, Ruschig, and Aumüller, *Angew. Chem.*, **52**, 363 (1939); Bockmühl, Ehrhart, and Ruschig, U. S. pat. 2,108,646.

[526] Marker and Krueger, *J. Am. Chem. Soc.*, **62**, 3349 (1940).

[527] Wettstein, *Helv. Chim. Acta*, **23**, 388 (1940).

Urane Derivatives. From the residues of the extracts of mares' pregnancy urine, Marker and co-workers [528] have isolated two compounds, which are formulated provisionally as $3(\beta)$,11-uranediol (LXXVIII) and $3(\alpha)$,11,20-uranetriol (LXXIX). Urane, m.p. 128°, the parent hydrocarbon, is regarded tentatively as being epimeric at C_9 with pregnane. The evidence for this isomerism comes largely from the study of uranetrione, the triketone from uranetriol. All the hydroxyl groups of urane-
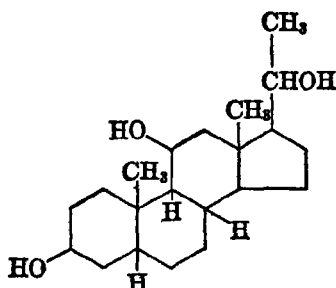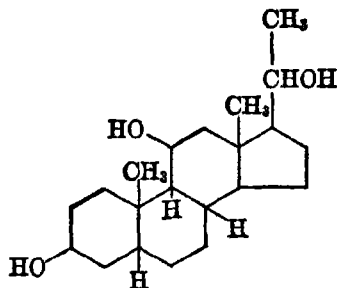
LXXVIII 3(β),11-Uranediol   LXXIX 3(α),11, 20-Uranetriol
(provisional)   (provisional)

triol can be acetylated, but one of the carbonyl groups of uranetrione is unreactive. This suggests attachment of the unreactive carbonyl group at C_{11} or C_{12}, as has been noted with other steroids (p. 1516). Uranetrione is not changed by boiling with acetic acid-hydrochloric acid, but is converted to a mixture on refluxing with methanolic alkali. The effect of the alkali may be to cause a partial epimerization of the C_9—H (*cf. allo*hyodesoxycholic acid, p. 1420). Consistent with this, when uranetrione is hydrogenated with platinum in acetic acid and the product oxidized with chromic acid, a mixture of 3,20-pregnanedione and uranedione, identical with the oxidation product of natural uranediol, is obtained. Since uranetriol does not and uranediol does give a precipitate with digitonin, opposite configurations of the C_3—OH groups are assigned.

Physiological Relationships of Progesterone. For several years after their isolation, progesterone and its enol acetate [529] were the only known compounds with progestational activity. It is now evident that certain modifications of the molecule may be made without destroying its physiological activity, but that retention of the C_3 carbonyl group in conjugation with the double bond at $C_4 : C_5$ is essential for potency. If the double bond at $C_4 : C_5$ is shifted to $C_1 : C_2$, to

$C_5 : C_6$, or to $C_{16} : C_{17}$, activity is completely destroyed.[530] With the structure of ring A constant, introduction of a double bond at C_6,[527] or of a 6(α?)-hydroxyl group (as acetate),[531] decreases the activity to one-half and one-third, respectively. The presence of an hydroxyl group at C_{11},[532] or of a carbonyl group at C_6[533] or at C_{11},[532] destroys activity. Modification of the C_{17} side chain leads to a variety of effects. Hydrogenation of the C_{20} carbonyl group destroys potency,[534] as does the introduction of an hydroxyl group at C_{17} [17(β)-hydroxyprogesterone],[535] but an hydroxyl group at C_{21} (21-hydroxyprogesterone acetate) does not destroy activity.[536] Shortening or lengthening the C_{17} side chain by a methyl group (20-norprogesterone and 21-methylprogesterone) decreases the activity to one-tenth, and lengthening by an ethyl group (21-ethylprogesterone) destroys activity completely.[537] Isomerization of the C_{17} side chain (isoprogesterone) likewise apparently destroys activity. Expansion of ring D into a six-membered ring (neoprogesterone, p. 1526), by incorporation of part of the C_{17} side chain with a shift of the carbonyl group to C_{17}, also destroys potency. Thus, the enol acetate of progesterone is the only compound with potency nearly equal to progesterone itself. In addition, testosterone and a number of 17-alkyltestosterones (p. 1505) have slight progestational activity. Important among these is 17-ethinyltestosterone, which has progestational potency when administered orally.[538]

In women, progesterone is formed solely by the corpus luteum during the second half of the menstrual cycle and during the first few months of pregnancy. After the second or third month of pregnancy, the placenta becomes the principal site of progesterone formation, and this transition is a critical phase in gestation.[539] With the development of the placenta as a source of the hormone, the amount of reduction product, 3(α),20(α)-pregnanediol, excreted in the urine gradually rises from 1–8 mg. per day to a level of 60–100 mg. per day at the end of preg-

[530] Butenandt and Mamoli, *Ber.*, **68**, 1850 (1935); Butenandt and Schmidt-Thomé, *Ber.*, **69**, 882 (1936); Butenandt, Mamoli, and Heusner, *Ber.*, **72**, 1614 (1939).

[531] Ehrenstein and Stevens, *J. Org. Chem.*, **5**, 318 (1940).

[532] Reichstein and Fuchs, *Helv. Chim. Acta*, **23**, 664 (1940).

[533] Ehrenstein, *J. Org. Chem.*, **4**, 506 (1939).

[534] Butenandt and Schmidt, *Ber.*, **67**, 2088 (1934).

[535] Pfiffner and North, *J. Biol. Chem.*, **132**, 459 (1940).

[536] de Fremery and Spanhoff, *Acta Brevia Neerland. Physiol. Pharmacol. Microbiol.*, **9**, 79 (1939) [C. A., **33**, 4299 (1939)].

[537] Miescher, Hunziker, and Wettstein, *Helv. Chim. Acta*, **23**, 1367 (1940); Wettstein, *ibid.*, **23**, 1371 (1940).

[538] Inhoffen *et. al.*, *Ber.*, **71**, 1024 (1938); Ruzicka, Hofmann, and Meldahl, *Helv. Chim. Acta*, **21**, 372 (1938); Serini and Köster, *Ber.*, **71**, 1766 (1938); Inhoffen and Köster, *Ber.*, **72**, 595 (1939); Hohlweg and Inhoffen, *Klin. Wochschr.*, **18**, 77 (1939).

[539] Review: Westphal, *Naturwissenschaften*, **30**, 461 (1940).

nancy.[540] The site of conversion of progesterone to pregnanediol is not certain, but usually the endometrium is regarded as an important tissue in the reduction.[541] In any event, after reduction pregnanediol glucuronide is excreted in the urine and may be extracted with butanol.[542] The origin of the other urinary compounds of Table XI and the form in which they are excreted are uncertain.

The Androgenic Hormones

From the urine of males, females, and eunuchs, and from testicular extracts, a number of hormones have been isolated which, when injected into castrated or immature males, bring about the restoration or development, respectively, of the latent secondary sexual characteristics. As with the estrogens, it was essential to have a biological method of assay for the isolation and study of these male hormones. The first feasible method was one developed by Gallagher and Koch,[543] in which the effect of the hormone on comb growth in capons is measured. At present the unit of activity (international comb unit) has been defined by international agreement as 0.1 mg. (100 μg.) of the male hormone, androsterone (Gr.: *andro*, male).[543] The assay is carried on in two groups of standardized capons, one with the standard hormone, the other with the substance under investigation. The growth of the comb is determined by means of a shadowgraph or by direct measurement of certain portions of the comb.

In a second method of assay, the effect on the accessory sex organs in castrated and immature rats (or mice) is measured. The assay is not as well defined as that on capons, and in this discussion only the values obtained by the methods of Butenandt and Tscherning[544] and of Tschopp[545] will be cited. In the Butenandt-Tscherning method immature rats (4 weeks old) are injected with a solution of the hormone in sesame oil for eight successive days, and on the ninth day a histological examination of the seminal vesicles is made. By varying the level at which the hormone is injected, that concentration which brings about development of the seminal vesicles comparable to that of control rats is

[540] Venning, *J. Biol. Chem.*, **126**, 597 (1938).

[541] Venning and Browne, *Am. J. Physiol.*, **123**, 209 (1938); Hamblen, Ashley, and Baptist, *Endocrinology*, **24**, 1 (1939); Buxton and Westphal, *Proc. Soc. Exptl. Biol. Med.*, **41**, 284 (1939).

[542] Venning and Browne, *Proc. Soc. Exptl. Biol. Med.*, **34**, 792 (1936); Odell and Marrian, *Biochem. J.*, **30**, 1533 (1936); Venning, *J. Biol. Chem.*, **119**, 473 (1937).

[543] Gallagher and Koch, *J. Pharmacol.*, **40**, 327 (1930). For review of the test, see Gustavson, Chapter XIV, in Allen, "Sex and Internal Secretions," 2nd ed., Williams and Wilkins Co., Baltimore (1939).

[544] Tscherning, *Ber.*, **68**, 679 (1935).

[545] Tschopp, *Arch. intern. pharmacodynamie*, **52**, 381 (1936).

determined. In the test of Tschopp, rats are castrated while immature and, after thirty days, are injected subcutaneously once a day for ten days with an oil solution of the hormone. At the end of the ten-day period, the animal is killed, and the seminal vesicles and the prostate are weighed. The daily level of hormone necessary for the development of a seminal vesicle weighing 40 mg. is determined either directly or by extrapolation. The results in both cases are expressed as rat units (R. U.), but the values from one assay are only qualitatively comparable to those of the other.

The natural androgens and their numerous transformation products are derivatives of etioallocholane (LXXX). This hydrocarbon was not known prior to the work on the androgenic hormones; it melts at 45–50° as against 79–80° for the isomeric etiocholane.[546] The physical properties and the physiological potencies of the important natural and derived androgens are given in Table XII.

Isolation of the Urinary Androgen. In 1931–32 Butenandt[547] reported the isolation of three compounds from the oil obtained by extracting male urine which previously had been boiled with hydrochloric acid. The substance obtained in largest quantity (20–25 mg.) was an hydroxyketone, $C_{19}H_{30}O_2$, to which the name androsterone was given. In analogy to the estrogenic hormones, Butenandt[548] suggested a struc-

LXXX. Etioallocholane
(Androstane)
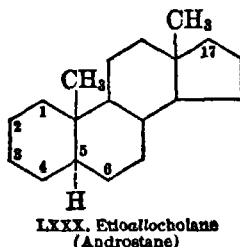
ture for androsterone, in which the functional groups were correctly distributed but in which the spatial configurations were in error. Plausibility was given to this suggestion by the discovery that a completely reduced estrone, octahydroestrone, had male hormone activity.[549] At this point, Ruzicka[550] converted the acetate of dihydrocholesterol, by

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[546] Butenandt and Tscherning, Z. physiol. Chem., **229**, 185 (1934); Reichstein, Helv. Chim. Acta, **19**, 979 (1936).

[547] Butenandt, Z. angew. Chem., **44**, 905 (1931); Angew. Chem., **45**, 655 (1932); Nature, **130**, 238 (1932).

[548] Butenandt, Wien. Klin. Wochschr., **47**, 935 (1934).

[549] Schoeller, Schwenk, and Hildebrandt, Naturwissenschaften, **21**, 286 (1933); Dirscherl and Voss, ibid., **22**, 315 (1934).

[550] Ruzicka, Goldberg, and Brüngger, Helv. Chim. Acta, **17**, 1389 (1934).

TABLE XII

ANDROGENIC HORMONES AND RELATED COMPOUNDS *

Hormone	Structural Modification of Etioallocholane †	Formula	M.P. °C.	$[\alpha]_D$ (alcohol)	Physiological Potency		
					μg./I.C.U.‡	μg./R.U.§	
						Tech ‖	B-T¶
			Diketones				
Δ¹-Androstenedione	3,17-Diketo-Δ¹-	$C_{19}H_{26}O_2$	150	+ 53.3°	ca. 150		
Δ⁴-Androstenedione	3,17-Diketo-Δ⁴-	$C_{19}H_{26}O_2$	173–174 c.	+185	130	78	500
Δ⁵-Androstenedione	3,17-Diketo-Δ⁵-	$C_{19}H_{26}O_2$	ca. 158		ca. 300		1500
Androstanedione	3,17-Diketo-	$C_{19}H_{28}O_2$	132 c.	+105	130	300	700
			Ketone Alcohols				
Dehydroandrosterone	3(β)-Hydroxy-17-keto-Δ⁵-	$C_{19}H_{28}O_2$	140–141 c.** 152–153 c.††	+ 10.9	ca. 200	ca. 500	3000
epi-Dehydroandrosterone	3(α)-Hydroxy-17-keto-Δ⁵-	$C_{19}H_{28}O_2$	223 c.	0	100	16	100
Testosterone	3-Keto-17(α)-hydroxy-Δ⁴-	$C_{19}H_{28}O_2$	154–154.5 c.	+109 (?)	15	>1000	
cis-Testosterone	3-Keto-17(β)-hydroxy-Δ⁴-	$C_{19}H_{28}O_2$	220–221 c.	+ 71.7	400		
Dihydrotestosterone	3-Keto-17(β)-hydroxy-	$C_{19}H_{30}O_2$	180–181 c.	+ 32.4	20		50
Androsterone	3(α)-Hydroxy-17-keto-	$C_{19}H_{30}O_2$	184–185 c.	+ 94.6	100	325	1000
Isoandrosterone	3(β)-Hydroxy-17-keto-	$C_{19}H_{30}O_2$	175–176	+ 89 (MeOH)	770		5000

Name	Configuration[†]	Formula	M.p.	[α]		
6-Oxotestosterone	3,6-Diketo-17-hydroxy-Δ⁴-	$C_{19}H_{26}O_3$	203–205	−58 (acetone)	inact.‡‡	50
Testosterone propionate	3-Keto-17-propionoxy-Δ⁴-	$C_{22}H_{32}O_3$	122–123		20	
Δ⁵-Testosterone acetate	3-Keto-17-acetoxy-Δ⁵-	$C_{21}H_{30}O_3$	130–147	−30.5	ca. 50	50
Dialcohols						
Δ⁴-Androstenediol	3(β),17(α)-Dihydroxy-Δ⁴-	$C_{19}H_{30}O_2$	155	−	200	750
Δ⁵-Androstenediol	3(β),17(α)-Dihydroxy-Δ⁵-	$C_{19}H_{30}O_2$	182 c.	−49.4	500	
cis-Δ⁵-Androstenediol	3(β),17(β)-Dihydroxy-Δ⁵-	$C_{19}H_{30}O_2$	198–198.5 c.		850–1000	95
Androstanediol	3(α),17(α)-Dihydroxy-	$C_{19}H_{32}O_2$	223 c.	+12.6	20	300
cis-Androstanediol	3(β),17(β)-Dihydroxy-	$C_{19}H_{32}O_2$	227–228 c.		350	
Isoandrostanediol	3(β),17(α)-Dihydroxy-	$C_{19}H_{32}O_2$	168 c.	+4	550	750
Methyl Derivatives						
Methyltestosterone	3-Keto-17(α)-hydroxy-17-methyl-Δ⁴-	$C_{20}H_{30}O_2$	163–164 c.	+82	25–30	
Methyldihydrotestosterone	3-Keto-17(α)-hydroxy-17-methyl-Δ⁵-	$C_{20}H_{32}O_2$	192–193 c.		20	22
17-Methylandrostanediol	3(α),17(α)-Dihydroxy-17-methyl-	$C_{20}H_{34}O_2$	184–185		35	
17-Methylisoandrostanediol	3(β),17(α)-Dihydroxy-17-methyl-	$C_{20}H_{34}O_2$	211–212 c.		500	

* Data largely from Goldberg, *Ergeb. Vitamin-Hormon/orsch.*, **1**, 371 (1938). Other sources: Dannenbaum, *Ergeb. Physiol.*, **38**, 796 (1936); Deanesly and Parkes, *Biochem. J.*, **20**, 291 (1936); Miescher and Klarer, *Helv. Chim. Acta*, **22**, 962 (1939); Butenandt and Dannenberg, *Ber.*, **73**, 206 (1940). The natural hormones are printed in bold-face type.

† Where the symbol Δ is used the ending "ane" should be changed to "ene."
‡ International comb units. § Rat units. ‖ Method of Tschopp. ¶ Method of Butenandt and Tscherning. ** Needles. †† Leaflets.
‡‡ Has estrogenic activity.

chromic acid oxidation, to etio*allo*cholan-3(β)-ol-17-one, and found it to be about one-seventh as potent as natural androsterone. Identification of androsterone as etio*allo*cholan-3(α)-ol-17-one (LXXXII) was soon accomplished by oxidizing *epi*dihydrocholesterol (LXXXI) with chromic acid.[551] At the same time the epimeric etiocholan-3-ol-17-ones were prepared from coprosterol and *epi*coprosterol and found to be inactive even
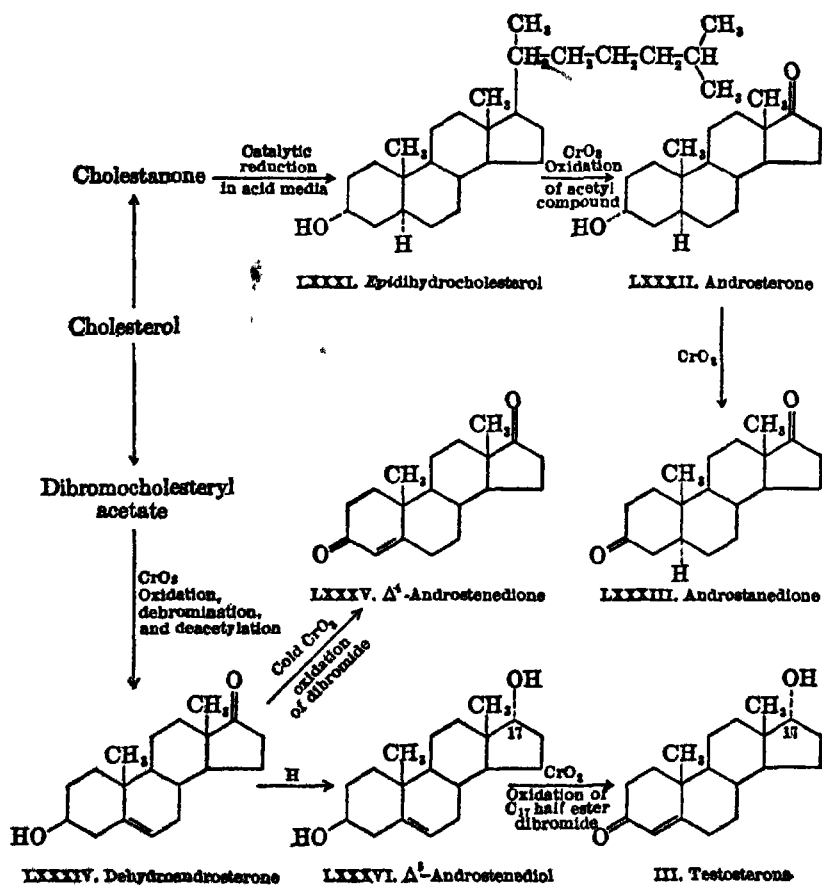
in doses fourteen times that of androsterone. Shortly after this work by Ruzicka, Butenandt [552] published the results of the examination of the structure of androsterone isolated from urine, and fully confirmed structure LXXXII.

The other two substances originally isolated from urine proved to be

[551] Ruzicka, Goldberg, Meyer, Brüngger, and Eichenberger, *ibid.*, **17**, 1395 (1934); Ruzicka, Goldberg, and Wirz, *ibid.*, **18**, 61 (1935).
[552] Butenandt and Tscherning, *Z. physiol. Chem.*, **229**, 167, 185 (1934).

dehydroandrosterone (LXXXIV) [Δ^5-etiocholen-3(β)-ol-17-one] and chlorodehydroandrosterone, formed by replacement of the C_3—OH group of dehydroandrosterone with chlorine.[553] The structure of dehydroandrosterone * was easily elucidated by application of the chromic oxide oxidation method to dibromocholesteryl acetate, followed by debromination of the oxidation product with zinc.[554]

A nearly inactive compound, etiocholan-3(α)-ol-17-one, is also present in urine.[555] It was first isolated as a corresponding 3,17-diol,[556] and it was some time before it was established that all the urinary androgens are 17-ketosteroids.† These three substances, androsterone, dehydroandrosterone, and etiocholan-3(α)-ol-17-one, are present in about the same quantities in male, female, and eunuch's urine.[555]

Testosterone. In the summer of 1935 the group of workers headed by Laqueur [557] presented convincing evidence that the male hormone obtained from testicular extract was different from those that had been isolated from the urine. This was apparent from the greater potency and from the fact that the potency of testicular extract was destroyed by treatment with alkali. A few milligrams of this very important compound was isolated and given the name testosterone. On examination, the testicular hormone proved to be an unsaturated ketone alcohol with strong absorption at 240 mμ (α,β-unsaturated ketone). The relationship of testosterone to progesterone was obvious, and it seemed probable that the two were structurally similar.

The structure of testosterone (III) was established by David [558] of Laqueur's group through its oxidation to Δ^4-androstene-3,17-dione (LXXXV), which is easily obtained from dehydroandrosterone by the standard procedures for the conversion of a Δ^5-en-3-ol to a Δ^4-en-3-one. This structure was confirmed by partial syntheses developed independ-

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[553] Butenandt and Grosse, *Ber.*, **69**, 2776 (1936); Wallis and Fernholz, *J. Am. Chem. Soc.*, **59**, 764 (1937).

* This compound is named transdehydroandrosterone by Ruzicka, since the C_3—OH group has the opposite configuration of the C_3—OH group of androsterone. Fieser has suggested the name dehydroisoandrosterone.

[554] Schoeller, Serini, and Gehrke, *Naturwissenschaften*, **23**, 337 (1935); Butenandt *et al.*, *Z. physiol. Chem.*, **237**, 57 (1935); Ruzicka and Wettstein, *Helv. Chim. Acta*, **18**, 986 (1935); Wallis and Fernholz, *J. Am. Chem. Soc.*, **57**, 1379, 1504 (1935).

[555] Callow and Callow, *Biochem. J.*, **33**, 931 (1939); **34**, 276 (1940).

[556] Ruzicka, Goldberg, and Bosshard, *Helv. Chim. Acta*, **20**, 451 (1937); Butenandt, Tscherning, and Dannenberg, *Z. physiol. Chem.*, **248**, 205 (1937).

† From the pregnancy urine of women, Marker *et al.*, *J. Am. Chem. Soc.*, **59**, 616, 768 (1937), isolated *allo*pregnan-3(α)-ol-20-one (*epiallo*pregnanolone) and reported it to have male hormone activity comparable to that of androsterone. Butenandt and Heusner, *Z. physiol. Chem.*, **256**, 236 (1938), have found that *epiallo*pregnanolone is without male hormone activity.

[557] David, Dingemanse, Freud, and Laqueur, *Z. physiol. Chem.*, **233**, 281 (1935).

[558] David, *Acta Brevia Neerland. Physiol. Pharmacol. Microbiol.*, **5**, 85, 108 (1935).

ently by Butenandt [559] and by Ruzicka [560] at nearly the same time; the transformation shown (p. 1502) is due to Ruzicka. By this method the acetate of dehydroandrosterone was reduced in the presence of nickel to the acetate of Δ^5-androstene-3,17-diol (LXXXVI). The product was benzoylated at C_{17} and then saponified at ca. 15°. In saponification, hydrolysis took place, preferentially at C_3, to give a C_{17} half ester, which was then converted to the ester of the α,β-unsaturated ketone either by direct oxidation with chromic acid or by oxidation of the 5,6-dibromo compound followed by debromination with zinc. The method of Butenandt was similar to that of Ruzicka, save that the diacetate rather than the mixed ester of Δ^5-androstene-3,17-diol was employed.*

Stereochemistry of the Hydroxyl Groups. As is evident from the physiological activities of androsterone and of isoandrosterone (Table XII), the configuration of the C_3—OH group has an important effect on the potency of the androgens. Even more pronounced is the effect on the potency of the configuration of a C_{17}—OH group. The mode of formation of Δ^5-androstenediol by catalytic reduction of dehydroandrosterone in neutral media indicates that the C_{17}—OH group is *trans* to the C_{13}—CH$_3$ group (v. Auwers-Skita rule, p. 1373) and, according to the convention used in this discussion, has an α-configuration. From the residues of large-scale preparation of Δ^5-androstenediol, Ruzicka [561] has isolated cis-Δ^5-androstenediol, the C_{17} epimer of Δ^5-androstenediol, and from this has prepared cis-testosterone. Both cis-Δ^5-androstenediol and cis-testosterone are much less potent than their epimers. Comparison of the physical properties (Table XII) of the two testosterones shows that testosterone has a lower melting point and a higher specific rotation than cis-testosterone. In accord with the assigned structures, the esters of testosterone are hydrolyzed more rapidly than those of cis-testosterone,[75] and testosterone is dehydrated less easily† than its

[559] Butenandt and Hanisch, *Ber.*, **68**, 1859 (1935); *Z. physiol. Chem.*, **237**, 89 (1935).

[560] Ruzicka and Wettstein, *Helv. Chim. Acta*, **18**, 1264 (1935); Ruzicka, Wettstein, and Kägi, *ibid.*, **18**, 1478 (1935).

*A second testicular hormone, m.p. 129–130°, and four inactive compounds from hogs' testes have been reported by Ogata and Hirano, *J. Pharm. Soc. Japan*, **54**, 199 (1934) [*C.A.*, **29**, 1871 (1935)], and by Hirano, *J. Pharm. Soc. Japan*, **56**, 717 (1936) [*C.A.*, **31**, 3125 (1937)], but apparently investigation of their structures has been discontinued.

[561] Ruzicka and Goldberg, *Helv. Chim. Acta*, **19**, 99 (1936); Ruzicka and Kägi, *ibid.*, **19**, 842 (1936).

†The difference in the ease of dehydration is shown by one of the color tests developed by Kägi and Miescher, reference 87. When cis-testosterone is heated in acetic acid solution with a trace of concentrated sulfuric acid, the solution cooled, and an acetic acid solution of bromine added, a bluish red color develops. With testosterone under the same conditions, no color is produced. Similar results are obtained with the other 17-hydroxysteroids: those with an α-configuration are resistant to mild dehydration and do not give colors, while those with a β-configuration readily give colors. Under more vigorous conditions of dehydration, both α- and β-forms are dehydrated, probably with the introduction of a double bond at C_{13}: C_{17} and with a migration of the C_{13}—CH$_3$ group to C_{17}.

epimer. With Δ⁵-androstenediol and testosterone as reference compounds, the steric configuration of hydroxyl groups at C_{17} of many other androstane derivatives has been established. As is shown in later transformations, other addition reactions taking place with C_{17} carbonyl groups lead to a predominance of the epimer with an α-configuration of the C_{17}—OH group.

Transformation of the Androgens. After the structures of the natural androgens had been determined, Ruzicka, Butenandt, and others investigated methods of producing variations in their molecular structures. Largely by the application of principles discussed in connection with the other steroids, saturated and unsaturated diketones,[562] ketone alcohols,[563] dialcohols,[564] and 17-alkyl derivatives[565] from etio*allo*cholane and etiocholane have been prepared, but will not be discussed in detail. For these transformations dehydroandrosterone and isoandrosterone are

[562] *3,17-Androstanedione:* Butenandt and Tscherning, *Z. physiol. Chem.*, **229**, 185 (1934); Ruzicka, Goldberg, and Meyer, *Helv. Chim. Acta*, **18**, 210 (1935). *3,17-Etiocholanedione:* Butenandt, Tscherning, and Dannenberg, *Z. physiol. Chem.*, **248**, 205 (1937). Δ¹-*Androstene-3,17-dione:* Butenandt and Dannenberg, *Ber.*, **73**, 206 (1940). *h*-Δ¹-*Androstene-3,17-dione:* Butenandt and Dannenberg, *Ber.*, **69**, 1158 (1936); Butenandt *et al.*, *Ber.*, **72**, 1617 (1939). Δ⁴-*Androstene-3,17-dione:* Ruzicka and Wettstein, *Helv. Chim. Acta*, **18**, 986 (1935); Butenandt and Kudzus, *Z. physiol. Chem.*, **237**, 75 (1935); Wallis and Fernholz, *J. Am. Chem. Soc.*, **57**, 1511 (1937); Oppenauer, *Rec. trav. chim.*, **56**, 137 (1937); Schoeller, Serini, and Logemann, U. S. pat. 2,175,220; Ruzicka and Wettstein, U. S. pat. 2,194,235. Δ⁵-*Androstene-3,17-dione:* Butenandt and Schmidt-Thomé, *Ber.*, **69**, 882 (1936). Δ⁴,⁶-*Androstadiene-3,17-dione:* Ruzicka and Bosshard, *Helv. Chim. Acta*, **20**, 328 (1937).

[563] *Androstan-3(α)-ol-17-one (Androsterone):* references 551 and 552. *Androstan-3(β)-ol-17-one (Isoandrosterone):* reference 550. *Androstan-17(α)-ol-3-one (Dihydrotestosterone):* Butenandt, Tscherning, and Hanisch, *Ber.*, **68**, 2097 (1935); Ruzicka and Goldberg, *Helv. Chim. Acta*, **19**, 99 (1936). *Androstan-17(β)-ol-3-one:* Ruzicka and Kägi, *ibid.*, **20**, 1557 (1937). Δ⁴-*Androsten-17(α)-ol-3-one (Testosterone):* references 559 and 560. Δ⁴-*Androsten-17(β)-ol-3-one (cis-Testosterone):* reference 561. Δ⁵-*Androsten-3(α)-ol-17-one (Epidehydroandrosterone):* Ruzicka and Goldberg, *Helv. Chim. Acta*, **19**, 1407 (1936). Δ⁵-*Androsten-3(β)-ol-17-one (Dehydroandrosterone):* references 554 and 566. Δ⁴,⁶-*Androstadien-17(α)-ol-3-one:* Ruzicka and Bosshard, *Helv. Chim. Acta*, **20**, 328 (1937); Wettstein, *ibid.*, **23**, 388 (1940).

[564] *3(α),17(α)-Androstanediol:* Ruzicka *et al.*, *Helv. Chim. Acta*, **18**, 210 (1935); Butenandt and Tscherning, *Z. physiol. Chem.*, **234**, 244 (1935). *3(α),17(β)-Androstanediol:* Ruzicka and Kägi, *Helv. Chim. Acta*, **20**, 1557 (1937). *3(β),17(α)-Androstanediol:* Ruzicka *et al.*, *ibid.*, **18**, 1487 (1935); Butenandt *et al.*, *Ber.*, **68**, 2097 (1935). *3(α),17(α)-Etiocholanediol:* reference 556. Δ⁵-*Androstene-3(α),17(α)-diol:* Ruzicka, Goldberg, and Bosshard, *Helv. Chim. Acta*, **20**, 541 (1937). Δ⁵-*Androstene-3(β),17(α)-diol:* Ruzicka and Wettstein, *ibid.*, **18**, 1264 (1935); Butenandt and Hanisch, *Ber.*, **68**, 1859 (1935); *Z. physiol. Chem.*, **237**, 89 (1935). Δ⁵-*Androstene-3(β),17(β)-diol:* Ruzicka and Kägi, *Helv. Chim. Acta*, **19**, 842 (1936).

[565] *17-Methyl derivatives:* Ruzicka *et al.*, *Helv. Chim. Acta*, **18**, 210, 1487 (1935); Miescher and Klarer, *ibid.*, **22**, 962 (1939). *17-Ethyl derivatives:* Ruzicka *et al.*, *ibid.*, **18**, 1487 (1935); **19**, 357 (1936); Butenandt *et al.*, *Ber.*, **71**, 1313 (1938). *17-Vinyl derivatives:* Serini and Logemann, *Ber.*, **71**, 1362 (1938); Ruzicka and Hofmann, *Helv. Chim. Acta*, **22**, 150 (1939). *17-Ethinyl derivatives:* Ruzicka and Hofmann, *ibid.*, **20**, 1280 (1937); Kathol, Logemann, and Serini, *Naturwissenschaften*, **25**, 682 (1937); Inhoffen, Logemann, Hohlweg, and Serini, *Ber.*, **71**, 1024 (1938).

the preferred intermediates, since they are more readily accessible than androsterone. Dehydroandrosterone is nearly always prepared from dibromocholesteryl acetate (*ca.* 3 per cent yield),[566] but it has been obtained also from other sterols and steroids.[567] Similarly, isoandrosterone is obtained from cholesterol *via* dihydrocholesterol. The preparation of androsterone from cholesterol presents the difficulty of producing an α-configuration of the C_3—OH group,[568] and because of this androsterone is seldom employed as an intermediate. Among other intermediates for the preparation of androsterone derivatives, Marker[569] has suggested the 17(α)-hydroxysteroids formed by the action of Caro's acid on the pseudosapogenins. (*Cf.* p. 1495).

A number of reactions involving 17-ketoandrostane derivatives are especially important because they provide methods for developing a side chain at C_{17}. By the action of alkyl Grignard reagents on the 17-ketoandrostanes, a mixture of isomers is obtained in which the 17(α)-hydroxy derivatives predominate.[570] Similarly, when hydrogen cyanide or alkali acetylides are employed, the 17(α)-hydroxy derivatives are the principal products.[571] The 17(α)-hydroxyandrostane derivatives exhibit the peculiarity of not forming insoluble digitonides when there is a free 3(β)-hydroxyl group present in the molecule.[572] On the other hand, the 17(β)-hydroxy derivatives form insoluble digitonides in the expected manner. Whether the inhibition of insoluble digitonide formation is due to the position of the hydroxyl group or to that of the side chain is uncertain, but the latter seems more probable in view of the behavior of the isopregnanes (p. 1493).

Using the cyanohydrin LXXXVII, obtained from dehydroandrosterone, Butenandt[573] has developed a method for the preparation of progesterone. The conversion is carried out by dehydrating the nitrile and reacting the dehydrated product LXXXVIII with methylmagnesium bromide. In this way, $\Delta^{5,16}$-pregnadien-3-ol-20-one (LXXXIX) is obtained, and from the latter progesterone is formed by reduction with Raney nickel (Δ^{16} hydrogenation) followed by oxidation and rearrangement by Oppenauer's method. Conversions of dehydroandrosterone to

[566] *Cf.* Butenandt *et al.*, reference 554, and Kiprianov and Frenkel, *J. Gen. Chem. (U.S.S.R.)*, **9**, 1682 (1939) [*C. A.*, **34**, 3756 (1940)].

[567] Oppenauer, *Nature*, **135**, 1039 (1935); Ruzicka, Fischer, and Meyer, *Helv. Chim. Acta*, **18**, 1483 (1935).

[568] *Cf.* Callow and Deansely, *Biochem. J.*, **29**, 1424 (1935), and Ruzicka, U. S. pat. 2,181,389, for preparative details.

[569] Marker *et al.*, *J. Am. Chem. Soc.*, **62**, 521, 650, 2543, 3003 (1940).

[570] *Inter al.*, Miescher and Klarer, *Helv. Chim. Acta*, **22**, 962 (1939).

[571] Miescher and Wettstein, *ibid.*, **21**, 1317 (1938).

[572] *Cf.* Reichstein and Gätzi, *ibid.*, **21**, 1185 (1938).

[573] Butenandt and Schmidt-Thomé, *Ber.*, **71**, 1487 (1938); **72**, 182 (1939).

LXXXIX. $\Delta^{1,16}$-Pregnadien-3-ol-20-one

LXXXVIII

LXXXVII

MeMgBr

POCl₃ and pyridine

HCN

LXXXIV. Dehydroandrosterone

EtMgBr

XC

POCl₃ and pyridine

XCI

OsO₄

XCII

Ac₂O (pyridine)

XCIII

Zn dist. dust

XCIV. Iso-Δ⁵-pregnenolone

Et α-chloro-propionate (NaOEt)

XCV

NaOH

XCVI + Δ⁵-Pregnenolone + Iso-Δ⁵-pregnenolone
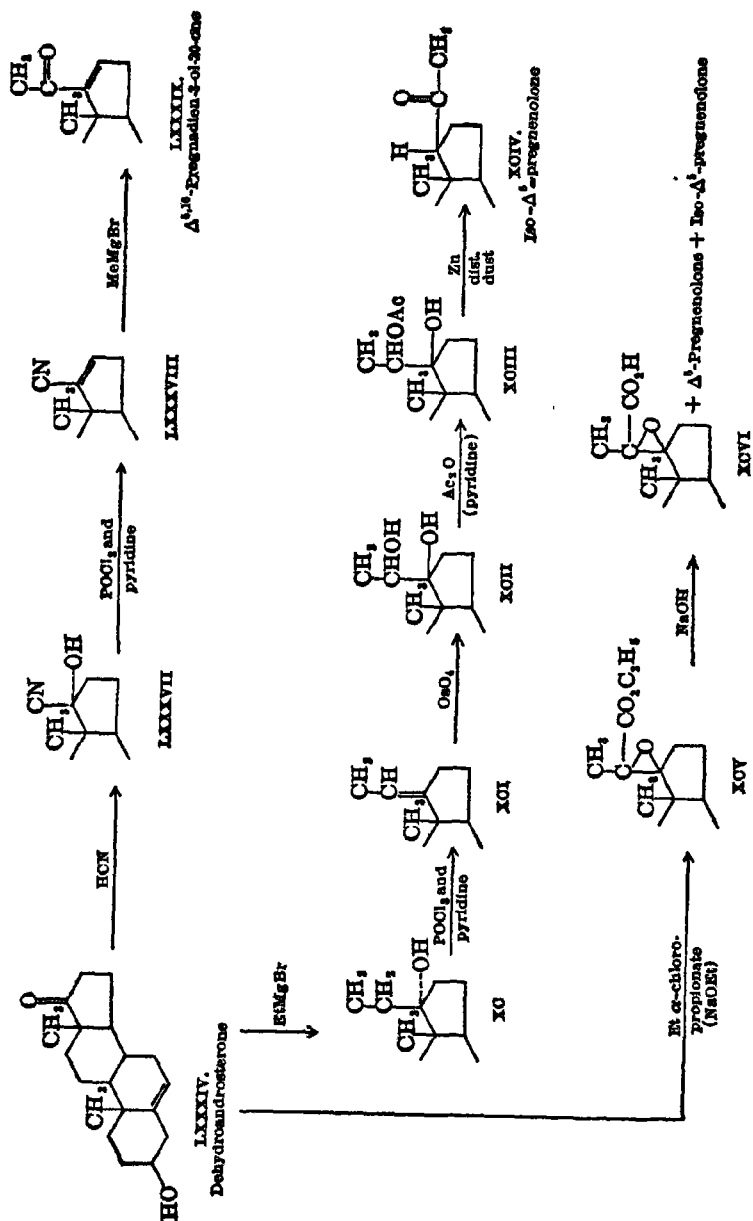
progesterone have been realized also by way of 17-ethylandrostenediol (XC),[574] or of 17-ethinylandrostenediol,[575] and by way of the reaction product of dehydroandrosterone and ethyl α-chloropropionate.[576] With the 17-ethyl derivative (XC), dehydration gives a product (XCI) which, on oxidation with osmium tetroxide, is converted to a glycol (XCII). The C_{21} acetate (XCIII) of the latter, on distillation with zinc dust in high vacuum,[577] is dehydrated and reduced to iso-Δ^5-pregnenolone (XCIV), and from this both isoprogesterone and progesterone are obtained. The hydration of the ethinyl derivative produces not only progesterone, but also neoprogesterone, and is discussed later in connection with homosteroids (p. 1526). With ethyl α-chloropropionate, dehydroandrosterone gives an ester oxide (XCV) which, on hydrolysis, is converted to the acid XCVI and a mixture of Δ^5-pregnenolone and iso-Δ^5-pregnenolone.

Only a limited amount of work on the conversion of the androgens to the estrogens has been done, but, by aromatization of ring A, Inhoffen [578] has converted androstan-17(α)-ol-3-one (XCVII) to α-estradiol (I). In the transformation, $\Delta^{1,4}$-androstadien-17(α)-ol-3-one acetate (XCVIIIa) was employed as an intermediate and was prepared by treating 2,4-dibromoandrostan-17(α)-ol-3-one acetate with collidine. After saponification of the ester, the free alcohol (XCVIIIb) was heated to 325°, and from the reaction mixture α-estradiol was obtained in ca. 50 per cent yield. Probably the first step in the thermal change is the migration of the C_{10}—CH_3 group to C_1 with simultaneous aromatization of ring A to form 1-methylestradiol (XCIX). As evidence of this the latter is formed when $\Delta^{1,4}$-androstadien-17(α)-ol-3-one is acted upon by a mixture of concentrated sulfuric acid and acetic anhydride.* In the pyrolysis, the C_1—CH_3 group of 1-methylestradiol is replaced by hydrogen, probably at the expense of the thermal product not accounted for. In passing, it may be noted that 1-methylestradiol is a weaker phenol than estradiol and is physiologically inactive.

Structure and Physiological Activity. Clinically, testosterone is the most important of the androgens. It is usually administered in the form

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[574] Butenandt, Schmidt-Thomé, and Paul, *Ber.*, **72**, 1112 (1939).

[575] Goldberg and Aeschbacher, *Helv. Chim. Acta*, **22**, 1185 (1939).

[576] Yarnall and Wallis, *J. Am. Chem. Soc.*, **59**, 951 (1937); U. S. pat. 2,123,217.

[577] Method of Slotta and Neisser, *Ber.*, **71**, 2345 (1938).

[578] Inhoffen, *Angew. Chem.*, **53**, 471 (1940); Inhoffen and Zühlsdorff, *Ber.*, **74**, 604 (1941).

* Parallels for the migration of the angular methyl group from C_{10} to C_1 are found in the conversion of santonin to desmotroposantonin [Clemo, Haworth, and Walton, *J. Chem. Soc.*, 2368 (1929); 1110 (1930); Clemo and Haworth, *ibid.*, 2579 (1930)], of $\Delta^{1,4}$-cholestadien-3-one to the cholesterol analog of 1-methylestradiol [Inhoffen and Huang-Minlon, *Naturwissenschaften*, **26**, 756 (1938)], and possibly of dianhydrostrophanthidin hemiacetal to trianhydrostrophanthidin (p. 1442).

of the propionate, since this ester, of the many that have been studied, seems to have the most prolonged action and the most favorable effect on seminal vesicle growth.[679] Because of the plurality of the biological effects, it is difficult to discuss structure and physiological action of the androgens. Korenchevsky and co-workers,[680] from a study of the effects

XCVII. Androstan-17(α)-ol-3-one acetate

Via 2,4-dibromo derivative →

Δ¹,⁴-Androstadien-17(α)-ol-3-one
XCVIIIa, R = Ac
XCVIIIb, R = H

conc. H₂SO₄ and Ac₂O

325°

XCIX. 1-Methylestradiol

L. α-Estradiol
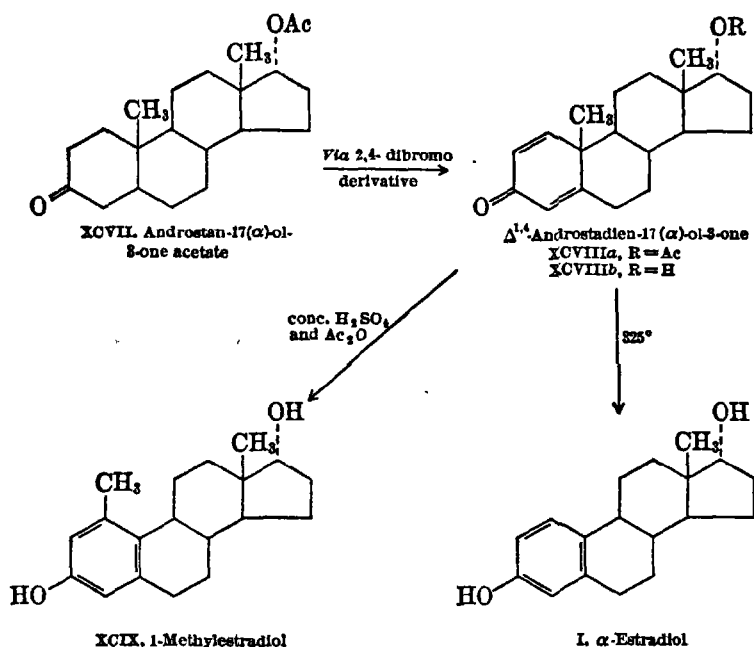
of the androgens and of mixtures of the androgens with other hormones on the entire genital system in both sexes, have concluded that, apart from progesterone, there are no purely "male" or "female" hormones. This is borne out by the distribution of estrone in urine, and by the fact that the same androgens are found in about equal quantity in the urine of males, females, and eunuchs.[656] According to Korenchevsky, the androgens may be divided into two groups: those with bisexual characteristics and those with chiefly male characteristics. To the bisexual group belong dehydroandrosterone, testosterone, and Δ⁵-androstenediol, since, in spite of their potency as androgens, they restore atrophied sexual organs in about the same degree in both males and females. Among the chiefly male hormones are androsterone, androstanediol,

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[679] Ruzicka and Wettstein, *Helv. Chim. Acta*, **19**, 1141 (1936); Miescher, Wettstein, and Tschopp, *Biochem. J.*, **30**, 1977 (1936); Miescher, Wettstein, and Schola, U. S. pat 2,109,400.

[680] Review: Korenchevsky, *Ergeb. Vitamin-Hormonforsch.*, **2**, 418 (1939).

testosterone propionate, and probably Δ^4-androstenedione. Of these, testosterone propionate is the most important because of its high activity and because of its extensive use clinically. Consideration of structure and physiological activity is further complicated by the issues of coöperative and antagonistic activities. In normal individuals a balance is maintained among the various hormones, certain characteristics resulting from coöperation and others from antagonism.

It is evident then that the values obtained for the activities of the androgens have no particular meaning unless considered in relation to the organism as a whole. This is extremely difficult. It is possible, however, to make some generalizations on the potency as determined by the special assays. From the data of Table XII, it is clear that the compounds related in structure to testosterone are the most potent androgens, and that this potency appears to be due largely to the presence of the carbonyl group at C_3 and unsaturation at C_4 in conjugation with this carbonyl group. Shifting the position of the unsaturation to $C_1 : C_2$ reduces the potency markedly. Given the favorable structure in ring A, the configuration about C_{17} has an important bearing on the activity, as is shown by the activities of testosterone and of *cis*-testosterone. Even though this favorable structure in ring A is retained, the introduction of alkyl groups at C_{17} modifies the physiological action so that both androgenic and progestational effects are manifested. Certain variations of structure in rings A and B bring about loss of androgenic potency and development of estrogenic activity. This is illustrated by the h-Δ^1-androstane derivatives,[581] formed by treating 2-bromoandrostanes with potassium acetate, by 6-oxotestosterone,[582] and by the 3-carboxyandrostan-17-ones,[583] all of which have moderate estrogenic activity.

THE ADRENAL SUBSTANCES

In the cortex of the adrenals are formed a number of steroid hormones which are essential to life. A deficit of these cortical substances (Addison's disease) produces bronzing of the skin, muscular weakness, a rise in blood urea, changes in the carbohydrate metabolism, and disturbances of the salt and water balance. Overproduction of the adrenal substances in children results in precocious sexual development, and this suggests a close relationship to the sex hormones.[*]

[581] *Cf.* Butenandt and Dannenberg, *Ber.*, **73**, 206 (1940).

[582] Butenandt and Riegel, *Ber.*, **69**, 1163 (1936).

[583] Marker *et al.*, *J. Am. Chem. Soc.*, **58**, 1948 (1936).

[*] For physiology see Grollman, "The Adrenals," Williams and Wilkins, Baltimore (1936), and Verzar, "Die Funktion der Nebennierenrinde," Schwabe, Basle (1939).

With the development of methods of obtaining crude potent extracts from the adrenals,[584] and of assaying these in adrenalectomized dogs [585] or rats,[586] it was thought at first that the potency was due to one hormone designated as cortin.[587] Later it became clear that the cortical activity was due to numerous closely related compounds. Six active crystalline compounds have been isolated from the adrenal extracts, but the potency of the crude extracts or of the amorphous residues is far greater than that of any of these.[588] The activity both of the pure compounds and of the extracts is defined in terms of cortin units. With dogs one cortin unit is the minimum daily dose per kilogram which, when administered over seven days, will maintain the blood-urea level and the weight of the animal in essentially normal condition.[585] With rats a fatigue test is used, but this method of assay is much less exact than the dog test.[586]

Isolation of the Adrenal Substances. In the isolation of the adrenal substances, the glands are extracted with alcohol or acetone, the solution freed of epinephrine, and the fats removed by partition between hydrocarbon solvents and water (or water-alcohol).[584] The resulting mixture is finally resolved by a combination of fractional crystallization, of partition between solvents, of chromatographic analysis, and of the use of special reagents such as Girard's reagent T (p. 1470).[589] The separation and isolation of the individual compounds are difficult because of their similarity and because of the very small amount of each present in the extracts.* Although Kendall and co-workers, and Wintersteiner and Pfiffner first developed the technique of isolating pure adrenal compounds, Reichstein has been far more successful in resolving the complicated mixture present in the extracts. Through the use of Girard's reagents, Reichstein was able to separate the extracts into ketonic and non-ketonic fractions, and subsequently was able to resolve these fractions largely by means of chromatographic analysis. In addition, many

[584] *Inter al.*, Swingle and Pfiffner, *Medicine*, **11**, 371 (1932); Grollman and Firor, *J. Biol. Chem.*, **100**, 429 (1933); Pfiffner and Vars, *ibid.*, **106**, 645 (1934); Cartland and Kuizenga, *ibid.*, **116**, 57 (1936); Waterman *et al.*, *Acta Brevia Neerland. Physiol. Pharmacol. Microbiol.*, **9**, 75 (1939); Uyldert, *Endocrinology*, **25**, 871 (1939).

[585] Pfiffner, Swingle, and Vars, *J. Biol. Chem.*, **104**, 701 (1934).

[586] Everse and de Fremery, *Acta Brevia Neerland. Physiol. Pharmacol. Microbiol.*, **2**, 152 (1932); Ingles, Hales, and Haslerund, *Am. J. Physiol.*, **113**, 200 (1935); Ingle, *ibid.*, **116**, 622 (1936).

[587] Hartmann and Brownell, *Am. J. Physiol.*, **97**, 530 (1931).

[588] Review: Kendall, *Arch. Path.*, **32**, 474 (1941).

[589] *Cf.* Reichstein, *Ergeb. Vitamin-Hormonforsch.*, **1**, 344 (1938).

* For example, from 20,000 steers about 1000 kilograms of adrenals is obtained. After extraction and purification, 18–60 grams of potent material, assaying about 1–2 million dog units, is isolated, and on resolution ca. 300 milligrams of each one of the more abundant compounds is separated.

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[581] Cf. Butenandt and Dannenberg, Ber., **73**, 206 (1940).

[582] Butenandt and Riegel, Ber., **69**, 1163 (1936).

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[585] Pfiffner, Swingle, and Vars, *J. Biol. Chem.*, **104**, 701 (1934).

[586] Everse and de Fremery, *Acta Brevia Neerland. Physiol. Pharmacol. Microbiol.*, **2**, 152 (1932); Ingles, Hales, and Haslerund, *Am. J. Physiol.*, **113**, 200 (1935); Ingle, *ibid.*, **116**, 622 (1936).

[587] Hartmann and Brownell, *Am. J. Physiol.*, **97**, 530 (1931).

[588] Review: Kendall, *Arch. Path.*, **32**, 474 (1941).

[589] *Cf.* Reichstein, *Ergeb. Vitamin-Hormonforsch.*, **1**, 344 (1938).

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TABLE XIII

PRINCIPAL ADRENAL SUBSTANCES *

Compound †	Position of Attachment of Substituents	Formula	M.P. °C.	[α]D (Alcohol)	Physiological Activity ‡	
					Rat Test (mg.)	Dog Test (mg.)
Allopregnane Derivatives						
Wintersteiner's A	3(β),11,17(β),20,21-Pentahydroxy-	$C_{21}H_{36}O_5$	221–222	+ 16°		
Wintersteiner's D	3(β),11,17(β),21-Tetrahydroxy-20-keto-	$C_{21}H_{34}O_5$	253–256 c.	+ 69		
Reichstein's D	3(β),17(β),21-Trihydroxy-11,20-diketo-	$C_{21}H_{32}O_5$	230–238 c.	+ 66		
Reichstein's K	3(β),17(β),20,21-Tetrahydroxy-	$C_{21}H_{36}O_4$	198–200 c.			
Reichstein's R	3(β),11,21-Trihydroxy-20-keto-	$C_{21}H_{34}O_4$	202–204 c.			
Reichstein's P	3(β),17(β),21-Trihydroxy-20-keto-	$C_{21}H_{34}O_4$	230–239 c.	+ 48		
Kendall's H	3(β),21-Dihydroxy-11,20-diketo-	$C_{21}H_{32}O_4$	189–191 c.			
Reichstein's J	3(β),17(β),20-Trihydroxy-	$C_{21}H_{36}O_2$	217–218 c.			
Reichstein's O	3(β),17(β),20-Trihydroxy-	$C_{21}H_{36}O_2$	222–223 c.			
Reichstein's L	3(β),17(β)-Dihydroxy-20-keto-	$C_{21}H_{34}O_3$	264–266 c.	+ 31		

Δ⁴-Pregnene Derivatives

Reichstein's E	3-Keto-11,17(β),20,21-tetrahydroxy-	$C_{21}H_{32}O_5$	ca. 125	+ 87		5.0
Reichstein's M	3,20-Diketo-11,17(β),21-trihydroxy-	$C_{21}H_{30}O_5$	207–210 c.	+167		1.25
Wintersteiner's F	3,11,20-Triketo-17(β),21-dihydroxy-	$C_{21}H_{28}O_5$	208	+209	2(I)	
Corticosterone	3,20-Diketo-11,21-dihydroxy-	$C_{21}H_{30}O_4$	180–182 c.	+222	0.5–1(E)	0.6–1.5
Reichstein's S	3,20-Diketo-17(β),21-dihydroxy-	$C_{21}H_{30}O_4$	200–206 c.			
Dehydrocorticosterone	3,11,20-Triketo-21-hydroxy-	$C_{21}H_{28}O_4$	177–180 c.	+299§	2(I)	
Desoxycorticosterone	3,20-Diketo-21-hydroxy-	$C_{21}H_{30}O_3$	141–142 c.	+178	0.8(E)‖	2.5
17(β)-Hydroxyprogesterone	3,20-Diketo-17(β)-hydroxy-	$C_{21}H_{30}O_3$	212–215	+102($CHCl_3$)		

Δ⁴-Androstene Derivatives

Adrenosterone	3,11,17-Triketo-	$C_{19}H_{24}O_3$	ca. 222 c.	+262		

* Data from Reichstein, Ergeb. Vitamin-Hormonforsch., 1, 334 (1938); from Pfiffner and North, J. Biol. Chem., 132, 459 (1940); and from Waterman et al., Acta Brevia Neerland. Physiol. Pharmacol. Microbiol., 9, 75 (1939).

† The names assigned, e.g., Wintersteiner's A, indicate priority in isolation

‡ The physiological activity is expressed in milligrams of substance required to keep an adrenalectomised animal in a normal condition. In the rat test the method of assay varies: (I) indicates an assay by the procedure of Ingle et al., Am. J. Physiol., 118, 200 (1935); 116, 622 (1936); (E) indicates the method of Everse and de Fremery, Acta Brevia Neerland. Physiol. Pharmacol. Microbiol., 2, 152 (1932).

§ [cide. ‖ Tested as acetate.

of the degradations and nearly all the partial syntheses establishing the structures of the adrenal compounds are due to Reichstein.

The principal known adrenal compounds are given in Table XIII, where they are identified partially by trivial names and partially by the alphabetic designations given them on isolation.* In the table the compounds are classified as derivatives of *allo*pregnane, of Δ^4-pregnene, and of Δ^4-androstene. The *allo*pregnane and Δ^4-pregnene groups are subdivided on the basis of oxygen content into $C_{21}O_5$, $C_{21}O_4$, and $C_{21}O_3$ derivatives. The potent compounds are derivatives of Δ^4-pregnene and are structurally similar to progesterone, which is present also in adrenal extracts and which has slight cortical activity.[588]

The known adrenal substances are polyhydroxy, hydroxyketonic, or polyketonic steroids.† Hydroxyl groups may be present at C_3, C_{17}, C_{20}, C_{21}, and probably at C_{11}. In the natural compounds, hydroxyl groups at C_3 and C_{17} always have a β-configuration, but hydroxyl groups at other positions have variable or unknown configurations. By a convention introduced by Reichstein, the C_{20}—OH group of many of the cortical substances is assumed arbitrarily to have a β-configuration.‡ Proof of the presence of an hydroxyl group at C_{11} in certain of the adrenal steroids is indirect, and because of this no consideration has been given to its steric configuration.

The *Allopregnane* Group. *The $C_{21}O_5$ Derivatives.* The first of the adrenal substances to be characterized was compound A of Wintersteiner's and Reichstein's series.[590] Through the formation of a tetra- and of a penta-acetate, compound A was known to possess five hydroxyl groups, one of which was less reactive than the others. Two of these hydroxyl groups were shown to be attached to adjacent carbon atoms, for compound A also forms a monoacetone derivative. Reichstein [591] then demonstrated that compound A was a C-glycerol derivative by studying the action of periodic acid and of lead tetraacetate. With periodic acid, formaldehyde and a dihydroxyketone (II) were obtained, and in the degradation two equivalents of oxygen were consumed, as is

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* The designations used by the principal investigators are not identical.

† The possibility that some might be aldehydic in nature was suggested by Kendall prior to the realization that the adrenal substances are steroids. No aldehydic compounds have been isolated from the extracts, and a number which have been prepared by Reichstein, *Helv. Chim. Acta* (1940–41), by partial synthesis appear to be inactive.

‡ This convention was proposed by Prins and Reichstein, *Helv. Chim. Acta*, **23**, 1490 (1940), using the configuration of Reichstein's compound J for reference, and has been developed further by v. Euw and Reichstein, *ibid.*, **24**, 401 (1941). A 20(*β*)-hydroxyl group as defined by Reichstein's convention is not necessarily identical with one defined by Marker's convention (p. 1491).

[590] Wintersteiner and Pfiffner, *J. Biol. Chem.*, **111**, 599 (1935); Reichstein, *Helv. Chim. Acta*, **19**, 29 (1936); Mason, Myers, and Kendall, *J. Biol. Chem.*, **114**, 613 (1936).

[591] Reichstein, *Helv. Chim. Acta*, **19**, 402 (1936).

characteristic for glycerol or C-glycerol derivatives.[592] By the action of chromic acid either on compound A or on the dihydroxyketone II, the triketone III was formed. The latter (III) on Clemmensen reduction was converted to a mixture of 17(α)-hydroxyandrostane and androstane.[593] Thus, the nucleus was characterized, and, through correlation with the other adrenal compounds, the nuclear structure of many of the members of the group was established.
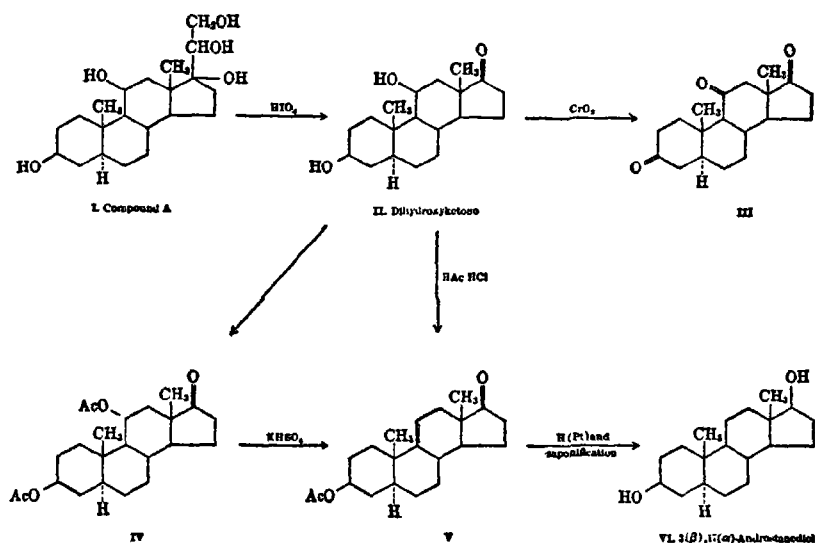
In the transformation given above, one of the hydroxyl groups of compound A could be provisionally assigned to C_3 through the fact that the dihydroxyketone II formed an insoluble digitonide. This view was strengthened when II was found to be weakly androgenic, and it was finally proved by the conversion of the dihydroxyketone to 3(β),17(α)-androstanediol (VI).[594] This change was effected by dehydration (loss of C_{11}—OH) of II to an unsaturated acetate (V), and catalytic reduction and hydrolysis of this to androstanediol. The dehydration was brought about either by heating with a solution of hydrogen chloride in acetic acid or by heating the diacetate (IV) of II with potassium acid sulfate.

Assignment to C_{11} of the hydroxyl group lost in the dehydration of the dihydroxyketone (II) follows from an indirect argument.[595] From

[592] Cf. Fischer and Dangschat, ibid., 17, 1196 (1934); 18, 1209 (1935).
[593] Reichstein, ibid., 19, 979 (1936).
[594] Schoppee, ibid., 23, 740 (1940).
[595] Steiger and Reichstein, ibid., 20, 817 (1937); Reichstein, ibid., 20, 978 (1937).

the evidence already presented one of the hydroxyl groups is attached at C_3, and the carbonyl group is located at C_{17}. The triketone formed by oxidation of the dihydroxyketone is not soluble in alkali, and one of the carbonyl groups is unreactive toward ketone reagents. The two reactive carbonyl groups are situated at C_3 and C_{17}, respectively, and positions C_1, C_2, C_4, C_{15}, and C_{16} are excluded for the unreactive carbonyl group since otherwise the triketone should have the properties of an α- or a β-diketone. Attachment at C_6 may be excluded through correlation with adrenosterone (p. 1520). Because of the unreactive nature of the unplaced carbonyl group, assignment to C_7 is excluded, and only attachment to C_{11} or C_{12} may be considered. Position C_{12} is improbable, since attachment at this position would lead to the manifestation of some of the properties of a β-diketone. Thus, C_{11} remains as the only position that is compatible with the reactions of the triketone. Further arguments for the attachment of an oxygen function at C_{11} in other adrenal substances are discussed later.

A β-configuration of the C_{17}—OH group of compound A and of other adrenal substances is indicated by the digitonide test.[596] Compound A and the other cortical compounds with hydroxyl groups at C_3 and C_{17} form insoluble digitonides. Since the 3(β)-hydroxyisopregnanes (p. 1494), and most of the 3(β),17(α)-dihydroxysteroids with a substituent at C_{17} (p. 1506), do not form insoluble digitonides, the positive test with the adrenal substances must indicate that both the C_3 and C_{17} hydroxyl groups have β-configurations. The evidence is admittedly tenuous.

Wintersteiner's compound D (VII) (Reichstein's C; Kendall's C) and Reichstein's compound D (VIII) (Kendall's G),[597] the other $C_{21}O_5$ derivatives, are represented provisionally by the formulas shown. The α-ketol structure of the side chains is indicated by the formation of formaldehyde and acid residues when either compound is treated with periodic acid. The distribution of the nuclear constituents is shown by the production of the triketone III when each compound is oxidized with chromic acid. Attachment of an oxygen function at C_{11} is not rigidly established in either case, but the nature of each grouping has been demonstrated by examining the acids formed when the C_{17} side-chain is oxidized away.

The $C_{21}O_4$ Derivatives. The $C_{21}O_4$ group comprises four compounds designated as Reichstein's K, P, and R, and as Kendall's H (Reichstein's

[596] Reichstein and Gätzi, *ibid.*, **21**, 1185 (1938); Miescher and Wettstein, *ibid.*, **22**, 112 (1939); Reichstein and Meystre, *ibid.*, **22**, 728 (1939).

[597] Wintersteiner and Pfiffner, *J. Biol. Chem.*, **111**, 599 (1935); Reichstein, *Helv. Chim. Acta*, **19**, 29, 402, 979 (1936); **20**, 978 (1937); Kendall *et al.*, *J. Biol. Chem.*, **119** (Proceedings), lvi (1937); Mason, Hoehn, and Kendall, *ibid.*, **124**, 459 (1938).

N). The structures of Reichstein's P (IX) and K (XI) were indicated by the early work in which they were oxidized by chromic acid to androstane-3,17-dione, or by periodic acid to isoandrosterone.[598] Through
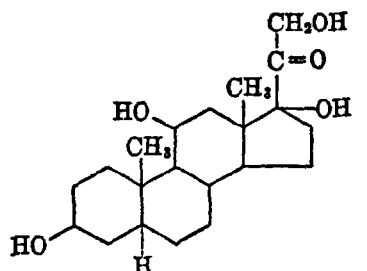
VII. Wintersteiner's D
[*Allo*pregnane-3(β),11,17(β),21-tetrol-20-one]
(provisional)
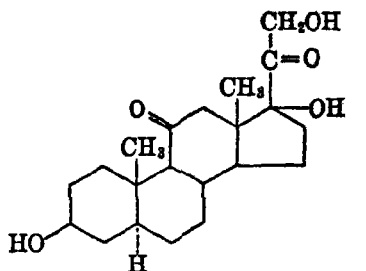
VIII. Reichstein's D
[*Allo*pregnane-3(β),17(β),21-triol-11,20-dione]
(provisional)

these oxidative degradations the presence of 3(β)-hydroxyl groups and of hydroxylated side chains was established. The structure of compound K was subsequently proved by partial synthesis * from Δ^{17}-*allo*pregnene-

IX. Reichstein's P

X

XI. Reichstein's K

XII. 17-Ethinylisoandrostane-3(β),17(α)-diol

XIII. 17-Ethinylisoandrostane-3(β),17(α)-diol

XIV. Δ⁹-Pregnene-3(β),21-diol
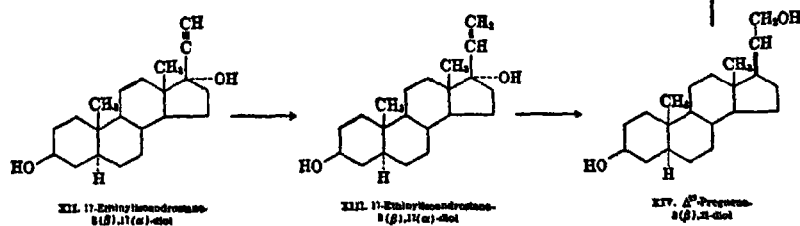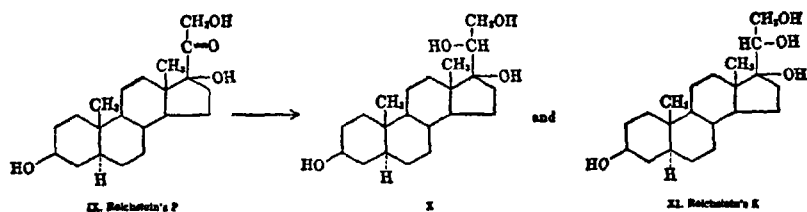
3,21-diol (XIV) through dihydroxylation with osmic acid.[599] The unsaturated diol was obtained in turn from 17-ethinylisoandrostane-3(β),

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[598] Steiger and Reichstein, *Helv. Chim. Acta*, **21**, 546 (1938); Reichstein and Gätzi, *ibid.*, **21**, 1185 (1938).

* A partial synthesis of Reichstein's P has been carried out by v. Euw and Reichstein, *Helv. Chim. Acta*, **24**, 401 (1941), from isoandrostane by a series of reactions analogous to those used for the partial synthesis of Reichstein's S (p. 1523).

[599] Serini, Logemann, and Hildebrand, *Ber.*, **72**, 391 (1939).

17(α)-diol (XII) by reduction to 17-vinylisoandrostane-3(β), 17(α)-diol (XIII) and rearrangement of the latter to XIV by treatment with acetic-trichloroacetic acid. The hydroxylation with osmic acid in this case appears to give a 17(β)-hydroxyl group exclusively.[600] The configuration of the hydroxyl group at C_{20} is also the same as that of the natural compound. This has been definitely established, since catalytic hydrogenation of Reichstein's P gives a mixture of the C_{20} isomers, X and XI.[598] In contrast the C_{20} isomers of the 17(α)-hydroxy derivatives are obtained as a mixture by the action of osmic acid on 17-vinylisoandrostane-3(β), 17(α)-diol and may be separated by chromatography.[599]

On oxidizing the diacetate of Reichstein's R, the diacetate of Kendall's H (Reichstein's N) is formed.[601] The structure of the latter was indicated first by Kendall through correlation with corticosterone and dehydrocorticosterone (p. 1521),[602] but it follows more directly by a transformation of Wintersteiner's A (I).[603] When the triacetate (XVII)

XV. Reichstein's R   XVI. Kendall's H

XVII. Wintersteiner's A triacetate   XVIII. iso-R diacetate
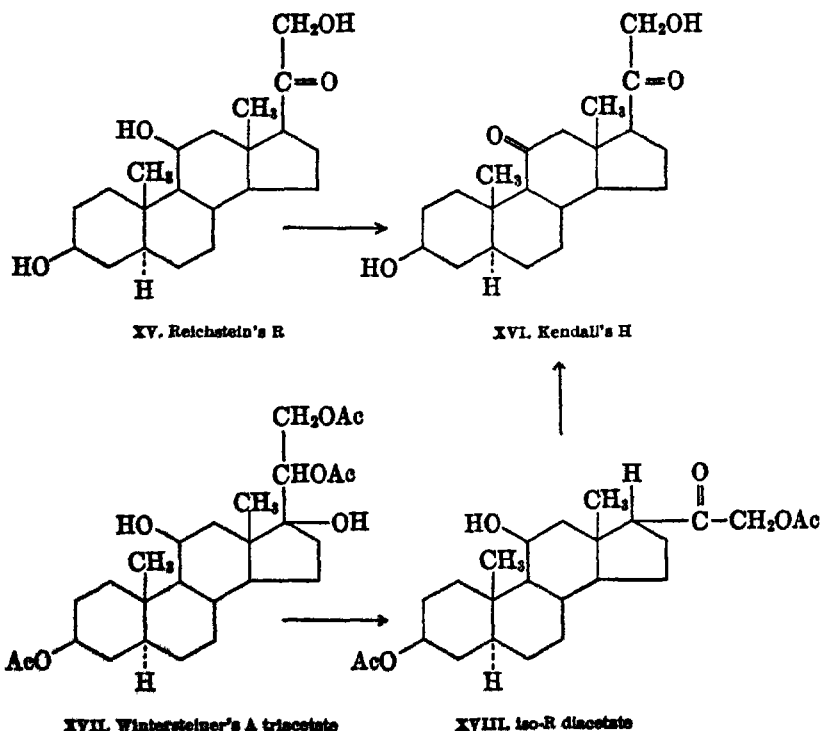
[600] *Cf.* Miescher and Wettstein, *Helv. Chim. Acta,* **22,** 112 (1939); Reichstein and Meystre, *ibid.,* **22,** 728 (1939).

[601] Reichstein and v. Euw, *ibid.,* **21,** 1197 (1938); Reichstein, *ibid.,* **21,** 1490 (1938).

[602] Mason, Hoehn, McKenzie, and Kendall, *J. Biol. Chem.,* **120,** 719 (1937).

[603] Shoppee and Reichstein, *Helv. Chim. Acta,* **23,** 729 (1940).

of compound A is heated with zinc dust in toluene, one molecule of acetic acid is eliminated and a strongly reducing compound (iso-R), which appears to be isomeric with Reichstein's R, is obtained as the acetate (XVIII) When this iso-R acetate is oxidized with chromic acid and the product rearranged with hydrochloric acid, the diacetate of compound H results. The rearrangement is comparable to the acid isomerization of isoprogesterone to progesterone (p. 1493).

The $C_{21}O_3$ Derivatives. Three trioxygenated *allo*pregnane adrenal substances are known. These compounds have been designated by Reichstein as substances L (XIX) (Wintersteiner's G), O (XX), and J (XXI).[604] The formulas shown were assigned originally on the basis of the analyses, of conversion to androstane derivatives by oxidative degradation, and of the formation of a mixture of compounds O and J

XIX. Reichstein's L   XX. Reichstein's O   XXI. Reichstein's J
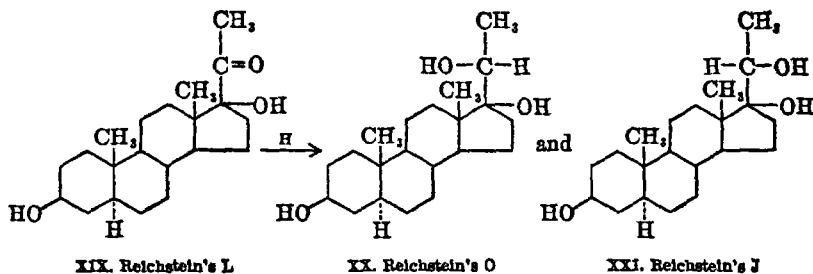
by catalytic reduction of L. The correctness of these formulations was established later by Reichstein [605] through hydroxylation of 3(β)-acetoxy-Δ^{17}-pregnene with osmic acid to a mixture of substances from which, after appropriate treatment, compound J was isolated as the principal product. An isomeric product was obtained also, in small amount, and this may be the 17(α)-hydroxy derivative.*

The Δ^4-Pregnene Group. The known substances with cortical activity are derivatives of Δ^4-pregnen-3-one. Of this group the most

[604] Reichstein, *ibid.*, **19**, 1107 (1936); Wintersteiner and Pfiffner, *J. Biol. Chem.*, **116**, 291 (1936); Steiger and Reichstein, *Helv. Chim. Acta*, **21**, 546 (1938); Reichstein and Gätzi, *ibid.*, **21**, 1497 (1938).

[605] Sutter, Meystre, and Reichstein, *Helv. Chim. Acta*, **22**, 618 (1939); Reich, Sutter, and Reichstein, *ibid.*, **23**, 170 (1940).

* In later work v. Euw and Reichstein, *Helv. Chim. Acta*, **24**, 418 (1941), have converted Reichstein's P (IX) to Reichstein's L by reacting the C_{20} carbonyl group of compound P with methylmagnesium bromide and oxidizing the product with periodic acid. Reichstein's J and O have been prepared from Reichstein's K (XI) by Prins and Reichstein, *ibid.*, **24**, 396 (1941), by a similar conversion. Compound K was oxidized with periodic acid to 17-formylandrostane-3(β),17(β)-diol, and the latter reacted with methylmagnesium bromide. The reaction product, after acetylation, was resolved by chromatographic analysis into acetates of substances J and O. According to an earlier paper with Prins, Reichstein, *ibid.*, **23**, 1490 (1940), suggests that the C_{17}—OH group of substances J and K be assigned a β-configuration, and that of substance O be regarded as α.

important is Δ^4-pregnen-21-ol-3,20-dione (desoxycorticosterone). All the members of the group show strong absorption in the ultra-violet at 240 mμ, and many give a characteristic greenish fluorescence when treated with concentrated sulfuric acid.[604] As with the *allo*pregnane derivatives, some of the compounds of this group are assigned an inert oxygen function at C_{11}, but the proof of the position of this function is indirect.

The $C_{21}O_5$ Derivatives. The group of compounds containing five oxygen atoms consists of Reichstein's E (XXII) [607] and M (XXIII) (Kendall's F) [608] and Wintersteiner's F (XXIV) (Kendall's E; Reichstein's Fa).[609] The position of the nuclear oxygen functions in all three
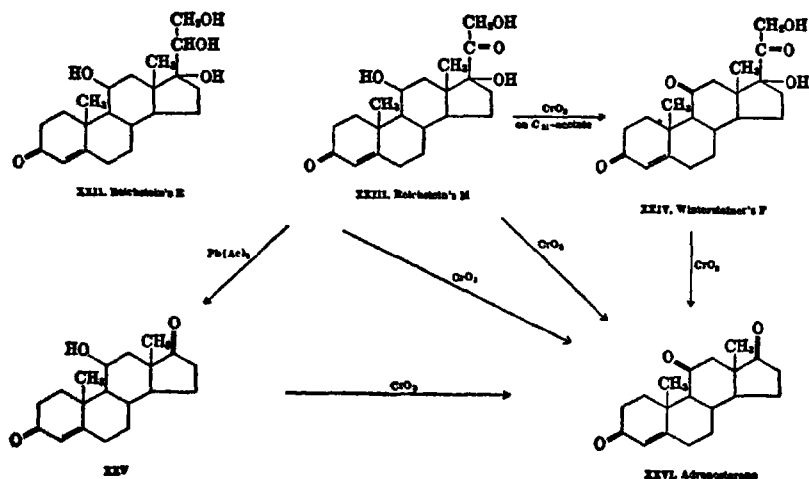
compounds has been determined, among other methods, by the conversion of each to adrenosterone (XXVI) by chromic acid oxidation. That Reichstein's E and Reichstein's M have hydroxyl groups at C_{11} is shown by the action of periodic acid or of lead tetraacetate. Reichstein's E, on treatment with periodic acid, is converted to an unsaturated hydroxydiketone (XXV), which, on oxidation with chromic acid, gives adrenosterone. Similarly, Reichstein's M gives the same diketone (XXV) when it is oxidized with lead tetraacetate. The position and

[604] Wintersteiner and Pfiffner, *J. Biol. Chem.*, **111**, 599 (1935); Reichstein, *Helv. Chim. Acta*, **19**, 1107 (1936); v. Euw and Reichstein, *ibid.*, **23**, 1114 (1940).

[607] Reichstein, *Helv. Chim. Acta*, **19**, 29 (1936); **20**, 453 (1937).

[608] de Fremery, Laqueur, Reichstein, Spanhoff, and Uyldert, *Nature*, **139**, 26 (1937); Reichstein, *Helv. Chim. Acta*, **20**, 953, 973 (1937); Mason, Hoehn, and Kendall, *J. Biol. Chem.*, **124**, 459 (1938).

[609] Wintersteiner and Pfiffner, *J. Biol. Chem.*, **111**, 599 (1935); **116**, 291 (1936); Mason, Myers, and Kendall, *ibid.*, **114**, 613 (1936); **116**, 267 (1936); Mason, Hoehn, and Kendall, *ibid.*, **124**, 459 (1938); Reichstein, *Helv. Chim. Acta*, **19**, 1107 (1936); **20**, 953 (1937).

nature of this C_{11} oxygen function are further shown by the fact that the monoacetate of Reichstein's M is converted by chromic acid oxidation into the monoacetate of Wintersteiner's F. The structure of the C_{17} side chain in each compound has been determined by studying the products obtained by periodic acid oxidation. Reichstein's E is slightly active as a cortical hormone and also has slight activity as an androgen. Reichstein's M and Wintersteiner's F are somewhat more potent than Reichstein's E as cortical substances.

The $C_{21}O_4$ *Derivatives.* Of the $C_{21}O_4$ derivatives, corticosterone (XXVII) (Kendall's B),[610] dehydrocorticosterone (XXVIII), and [611] Reichstein's S (XXXVI) [612] have been isolated in crystalline form. A
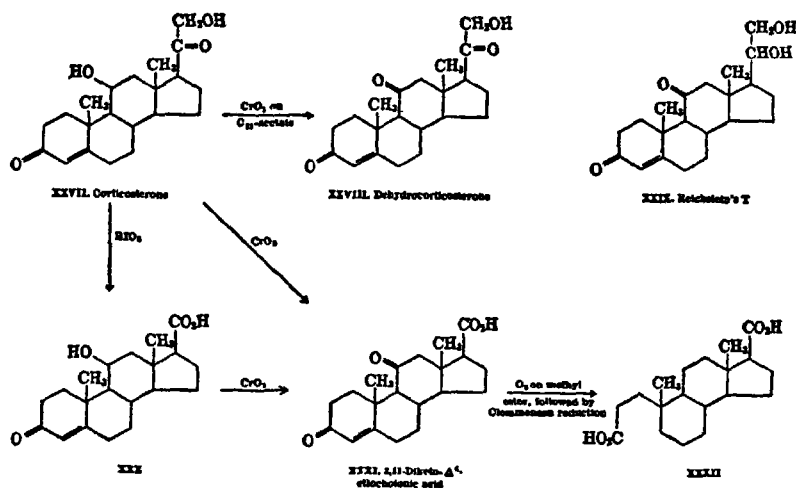
fourth compound, Reichstein's T (XXIX), has been obtained only in the form of its acetate.[613] On oxidation of corticosterone with chromic acid 3, 11-diketo-Δ^4-etiocholenic acid (XXXI) is formed. If periodic acid is substituted for chromic acid, 3-keto-11-hydroxy-Δ^4-etiocholenic acid (XXX) is obtained, and from this, by chromic acid oxidation, the diketo acid is easily formed. The structure of the diketo acid has been determined by comparing it with 3,12-diketo-Δ^4-etiocholenic acid, prepared by

[610] de Fremery, Laqueur, Reichstein, Spanhoff, and Uyldert, *Nature,* **139,** 26 (1937); Reichstein, *ibid.,* **139,** 331 (1937); Steiger and Reichstein, *ibid.,* **139,** 925 (1937); Reichstein, U. S. pat., 2,166,877; Kendall, Mason, Hoehn, and McKenzie, *Proc. Staff Meetings Mayo Clinic,* **12,** 136, 270 (1937); Mason, Hoehn, McKenzie, and Kendall, *J. Biol. Chem.,* **120,** 719 (1937).

[611] Mason, Myers, and Kendall, *J. Biol. Chem.,* **114,** 613 (1936); Reichstein and v. Euw, *Helv. Chim. Acta,* **21,** 1197 (1938).

[612] Reichstein and v. Euw, *Helv. Chim. Acta,* **21,** 1197, 1490 (1938).

[613] Reichstein and v. Euw, *ibid.,* **22,** 1222 (1939).

the degradation of desoxycholic acid (p. 1363). In this important work Mason and Hoehn [614] found that the product from the bile acid differed widely in its physical properties from that obtained from corticosterone. Attempts to provide a direct proof by synthesizing 3,11-diketo-Δ^4-etiocholenic acid have failed. The structure of the C_{17} side chain of corticosterone follows from the course of the periodic acid oxidation given above. Since formaldehyde is simultaneously formed with the 3-keto-11-hydroxy-Δ^4-etiocholenic acid, the side chain must have the structure shown. The structure of dehydrocorticosterone follows from the fact that the monoacetate of corticosterone, on treatment with chromic acid, is converted to the monoacetate of dehydrocorticosterone.

Using the diketoetiocholenic acid (XXXI) from corticosterone, Reichstein [615] has provided a proof of the position of the double bond at C_4 and of the carbonyl group at C_3 in corticosterone and in dehydrocorticosterone. By ozonization of the methyl ester of this acid, the unsaturated ring is opened and a ketodicarboxylic acid is obtained. Clemmensen reduction converts this ketodicarboxylic acid to a dicarboxylic acid (XXXII), which is identical with the acid obtained by similar treatment from 3-keto-Δ^4-etiocholenic acid or from progesterone.

At the time of its isolation the structure of Reichstein's S (XXXVI) was apparent since it reduced alkaline silver diammine solutions in the cold (α-ketol group), and since it was converted to Δ^4-androstene-3,17-dione by chromic acid oxidation.[612] This structure, together with the steric configuration of the C_{17}—OH group, has been established by two partial syntheses due to Reichstein. If allyltestosterone is dehydrated, and the product hydroxylated by treatment with osmic acid, α,β,γ-trihydroxypropyltestosterone is formed.[616] By temporarily protecting the C_{21} and the C_{22} hydroxyl groups by reaction with acetone, the C_{20} hydroxyl group may be acetylated, and after removal of acetone XXXII is obtained.[617] Periodic acid oxidation of the latter converts it to the aldehyde acetate XXXIII. On mild hydrolysis with potassium bicarbonate Δ^4-pregnene-17,20-diol-3-on-21-al (XXXIII) is formed, and this is readily rearranged by boiling with pyridine [618] to Reichstein's S.[619] The β-configuration of the C_{17} hydroxyl group was shown by periodic acid oxidation of compound S to 3-keto-17(β)-hydroxy-Δ^4-etiocholenic acid. The latter was characterized through its methyl ester (XXXVII),[620]

[614] Mason and Hoehn, J. Am. Chem. Soc., 60, 2566 (1938).
[615] Reichstein and Fuchs, Helv. Chim. Acta, 23, 676 (1940).
[616] Butenandt and Peters, Ber., 71, 2688 (1938).
[617] v. Euw and Reichstein, Helv. Chim. Acta, 23, 1114 (1940).
[618] Method: Fischer et al., Ber., 60, 479 (1927).
[619] Reichstein and v. Euw, Helv. Chim. Acta, 23, 1258 (1940).
[620] Reichstein, Meystre and v. Euw, ibid., 22, 1107 (1939).

which was prepared for comparison from methyl 3(β),17(β)-dihydroxy-Δ⁵-etiocholenate (XXXVIII) by the Oppenauer reaction.[621]
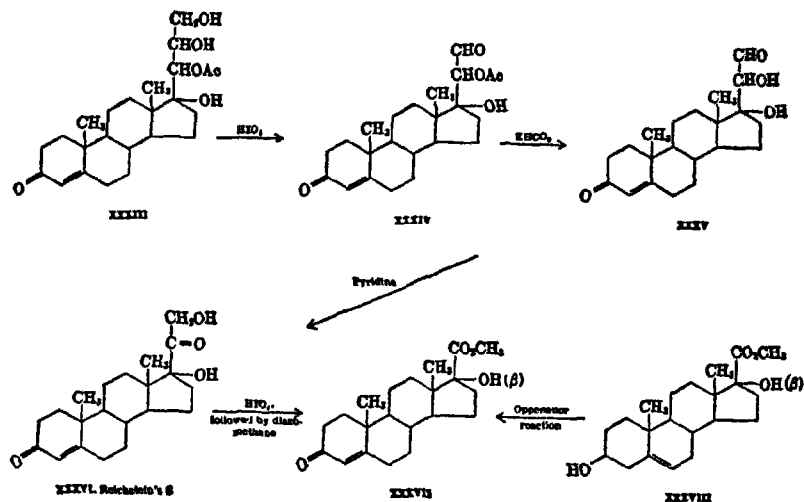
The C₂₁O₃ Derivatives. Desoxycorticosterone (XLIV) and 17(β)-hydroxyprogesterone are trioxygenated Δ⁴-pregnene derivatives. Reichstein[622] prepared the former by partial synthesis from 3-acetoxy-Δ⁵-

cholenic acid (XXXIX), as shown in the transformation XXXIX-XLIV, about a year prior to its isolation from adrenal extracts.[622]
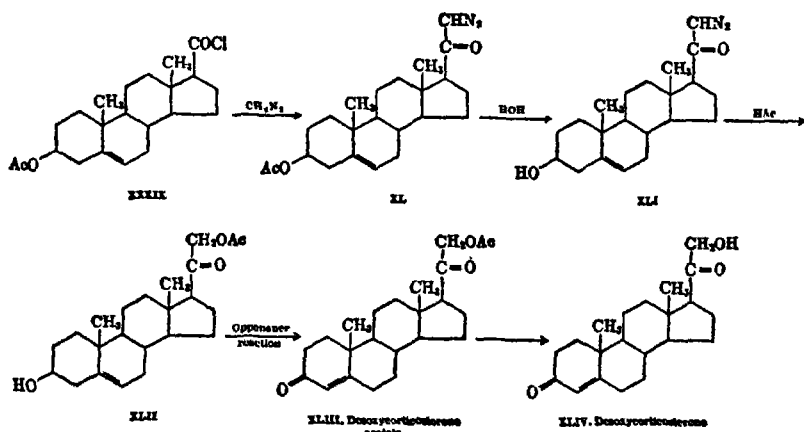
[621] Reichstein and Meystre, *ibid.*, **22**, 728 (1939).

[622] Steiger and Reichstein, *Nature*, **139**, 925 (1937); *Helv. Chim. Acta*, **20**, 1164 (1937); Reichstein and v. Euw, *ibid.*, **23**, 136 (1940).

[623] Reichstein and v. Euw, *Helv. Chim. Acta*, **21**, 1197 (1938).

Desoxycorticosterone may be obtained in poor yield by the action of lead tetraacetate on progesterone,[624] and of Caro's acid on certain of the pseudosapogenins (p. 1494). The tosyl derivatives of desoxycorticosterone and of related ketols are unusually reactive. For example, the tosyl group can be replaced with iodine by heating the tosyl derivatives with sodium iodide in acetone. The iodine may then be replaced with hydrogen, and in this way the C_{21} hydroxyl group has been reductively replaced in a number of instances.[625] Isodesoxycorticosterone and its acetate have been prepared by a procedure similar to that used for the preparation of iso-R acetate.[626] Both isodesoxycorticosterone and its acetate are physiologically inactive.

An inactive compound, 17(β)-hydroxyprogesterone,* has been isolated from the adrenals [627] and prepared by partial synthesis [628] as shown in formulas XLV–XLVII. The intermediate XLV was obtained by rearranging 17-vinyltestosterone by the process

$$\begin{array}{c} OH \\ | \\ -C-CH=CH_2 \end{array} \xrightarrow{PBr_3} \ >C=CH-CH_2-Br \rightarrow \ >C=CH-CH_2OAc$$
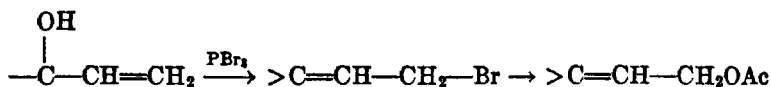
and dihydroxylating the end product with osmium tetroxide. As brought out in the partial synthesis of Reichstein's K (p. 1517), the hydroxylation of the semicyclic double bond leads almost exclusively to a β-configuration of the C_{17}—OH group. The 17 (α)-hydroxy isomer of XLV has been oxidized with periodic acid, and the product treated with diazomethane. In contrast, an oxido derivative rather than a ketone was obtained. 17(β)-Hydroxyprogesterone has no cortical or progestational activity, but does have androgenic potency about equal to that of androsterone.

The Δ^4-Androstene Group. Adrenosterone (XXVI) is the only member of the Δ^4-androstene group that has been isolated from the cortical extracts.[629] Its structure as Δ^4-androstene-3,11,17-trione fol-

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[624] Erhart, Ruschig and Aumüller, *Ber.*, **72**, 2035 (1939); Reichstein and Montigel, *Helv. Chim. Acta*, **22**, 1212 (1939).

[625] Reichstein and Schindler, *Helv. Chim. Acta*, **23**, 669 (1940); Reichstein and Fuchs, *ibid.*, **23**, 684 (1940).

[626] Shoppee, *ibid.*, **23**, 925 (1940).

* A compound described as 17(α)-hydroxyprogesterone was obtained by Ruzicka and Meldahl, *Helv. Chim. Acta*, **21**, 1760 (1938); **22**, 421 (1939) by rearranging the hydration product of the acetylene addition compound from dehydroandrosterone. This was subsequently recognized by Ruzicka, reference 635, as a D-homosteroid (p. 1526). The so-called 17(α)-hydroxyprogesterone has been reported to have cortical activity.

[627] Pfiffner and North, *J. Biol. Chem.*, **132**, 459 (1940); v. Euw and Reichstein, *Helv. Chim. Acta*, **24**, 879 (1941).

[628] Prins and Reichstein, *Helv. Chim. Acta*, **24**, 945 (1941).

[629] Reichstein, *Helv. Chim. Acta*, **19**, 29 (1936).

lows from its conversion to the triketone III on catalytic hydrogenation with palladium as a catalyst.[630] Δ^4-Androsten-11-ol-3,17-dione (XXV), the product of periodic acid oxidation of Reichstein's E, may be classed also as a member of this group although it has not been noted in nature. Controlled catalytic hydrogenation of either of these compounds has given a variety of 3,11,17-trioxygenated androstane derivatives.[630] None of this group has cortical activity, but all are weakly androgenic in the comb test; adrenosterone, for example, has about one-fifth the androgenic potency of androsterone.

XLV. Δ^4-Pregnene- 17 (β), 20 (β),21-triol-3-one   XLVI. 17-Formyl-Δ^4- androsten-17 (β)-ol-3-one   XLVII. 17 (β)- Hydroxyprogesterone
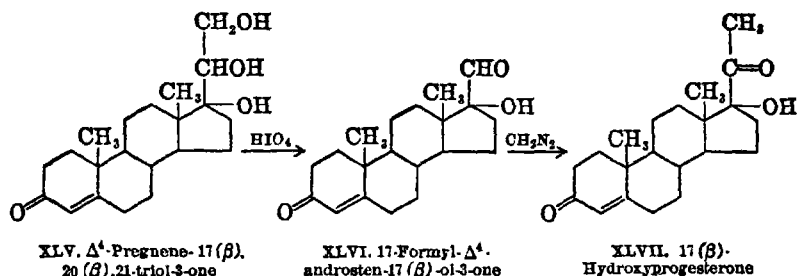
Structure and Physiological Activity. The relation of structure and physiological activity of the steroid adrenal hormones has been summarized by Kendall.[583] The three known physiological processes affected by the hormones are: (1) the permeability and transfer of electrolytes and water; (2) the activity of tissues with specialized functions, e.g., liver, kidney, etc.; and (3) the activation of enzymes. The amorphous residue and those compounds without an oxygen function at C_{11}, such as desoxycorticosterone, seem to be particularly effective in regulating electrolyte and water balance and in controlling the activity of the tissues. In contrast, the adrenal substances oxygenated at C_{11} are necessary for the activation of enzymes, especially those involved in carbohydrate metabolism. The effects are so manifold and so little understood, however, that definite correlations cannot be made. Because of this, the values for physiological activity given in Table XIII are more in the nature of physiological constants than of true representations of physiological potency.

[630] Reichstein, ibid., 19, 1107 (1936); Steiger and Reichstein, ibid., 20, 817 (1937).

THE HOMOSTEROIDS

In 1938, Miescher and Kägi [681] applied Darzens' synthesis [682] to dehydroandrosterone acetate (I) with the expectation of obtaining Δ^5-pregnenolone acetate (III), and from this progesterone. This was realized, but there was obtained also a product isomeric with Δ^5-pregnenolone acetate which was designated as neopregnenolone acetate (IV), and which gave neoprogesterone, an isomer of progesterone.[*] In the formation of neopregnenolone acetate, enlargement of ring D from a five- to a six-membered ring occurs. This was shown by Ruzicka [633] more clearly by a study of the reactions of the 17(α)-hydroxy-17-ethinyl derivative (V) of dehydroandrosterone acetate. With Nieuwland's method [634] of hydrating with boron trifluoride and mercuric oxide in acetic acid, ring enlargement occurred, and the product VII was converted to neopregnenolone by treatment with phosphorous tribromide ($C_{17\alpha}$—OH → $C_{17\alpha}$—Br) followed by debromination with zinc and acetic acid. That ring D is six-membered in neopregnenolone has been established by Ruzicka and Meldahl [635] through conversion to the corresponding saturated diol and dehydrogenation of this product with selenium to 1-methylchrysene, characterization of the hydrocarbon being made with a synthetic product.[636] As evidence that ring enlargement occurs during hydration, both the hydration product and neopregnenolone have been converted to the same saturated hydrocarbon, 17a-methyl-D-homoandrostene (cf. formula XI).[637] The mechanism of the reaction is not entirely clear, but Stavely [638] has shown that in the presence of aniline and with mercuric chloride as a catalyst an intermediate in the reaction is the 17-hydroxy-20-one derivative (VI), which is rearranged by alkali to a six-membered ring. According to Reichstein [627] and Stavely, the rearrangement takes place whether the C_{17} hydroxyl group has an α- or a β-configuration.

[681] Miescher and Kägi, *J. Soc. Chem. Ind.*, **57**, 276 (1938); *Helv. Chim. Acta*, **22**, 184 (1939).

[632] Darzens, *Compt. rend.*, **203**, 1374 (1936); Darzens and Levy, *ibid.*, **204**, 272 (1937). In this synthesis a ketone is caused to react with an α,α-dihalogenated aliphatic ester in the presence of magnesium amalgam.

[*] At first neoprogesterone was reported to be biologically equal to progesterone, but later it was found by Wettstein, reference 527, footnote 6, to be inactive even in doses of 30 mg.

[633] Rusicka and Meldahl, *Helv. Chim. Acta*, **22**, 421 (1939); cf. Ruzicka, Gätzi, and Reichstein, *ibid.*, **22**, 626 (1939); Ruzicka and Hunziger, *ibid.*, **22**, 707 (1939); Stavely, *J. Am. Chem. Soc.*, **61**, 79 (1939); *ibid.*, **62**, 489 (1940); *ibid.*, **63**, 3127 (1941).

[634] Hennion, Killian, Vaughn, and Nieuwland, *J. Am. Chem. Soc.*, **58**, 1130 (1936).

[635] Rusicka and Meldahl, *Helv. Chim. Acta*, **23**, 364 (1940).

[636] Rusicka and Markus, *ibid.*, **23**, 385 (1940).

[637] Rusicka and Meldahl, *ibid.*, **22**, 513 (1940).

To obtain products without a methyl group at position 17a the Tiffeneau [638] reaction has been applied to dehydroandrosterone acetate and to estrone.[639] In these transformations a cyanohydrin is formed on the C$_{17}$ carbonyl group, the product reduced to the corresponding amine, and the amine deaminated and rearranged by treatment with

I. Dehydroandrosterone acetate

II. Hydrolysis, and decarboxylation in quinoline

III. Δ⁵-Pregnenolone acetate

IV. Neopregnenolone acetate

X. D-Homodehydroandrosterone acetate
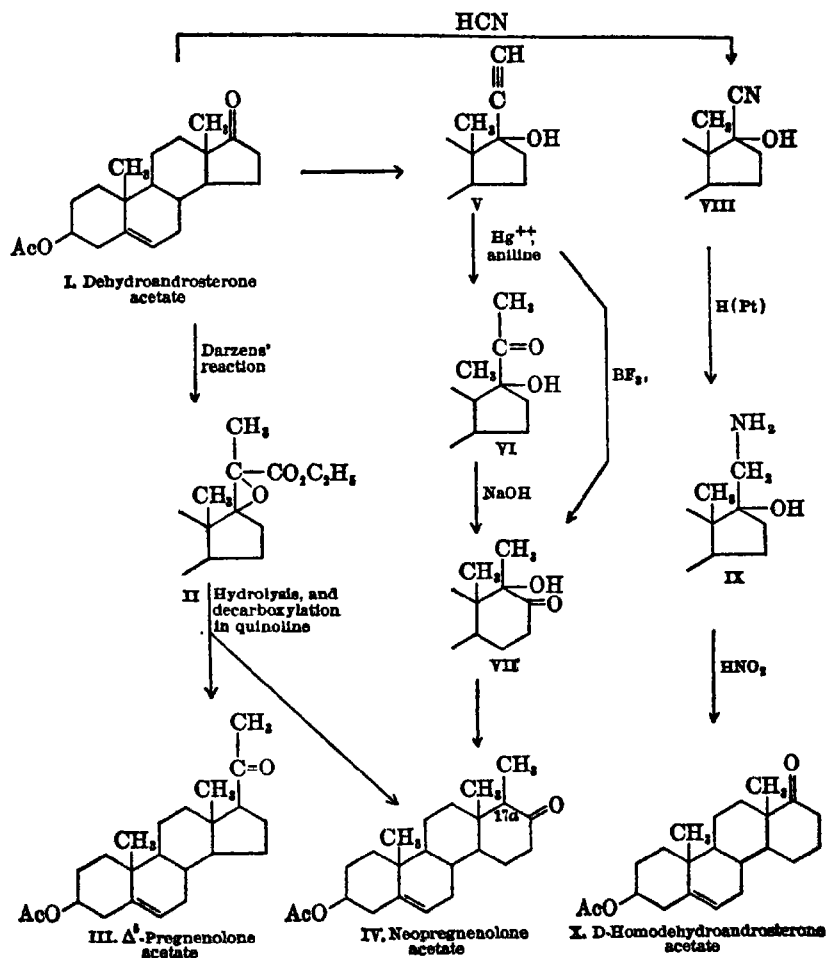
nitrous acid. The change is illustrated by the conversion of dehydroandrosterone acetate (I) to the cyanohydrin (VIII), reduction of the latter to the amine (IX), and finally deamination and rearrangement to D-homodehydroandrosterone acetate (X). By standard methods the

[638] Tiffeneau, Weill, and Tchoubar, *Compt. rend.*, **205**, 54 (1937).

[639] Goldberg and Monnier, *Helv. Chim. Acta*, **23**, 376, 840 (1940); Goldberg and Studer, *ibid.*, **24**, 478 (1941).

D-homosteroids have been obtained from the various intermediates. Ring enlargement in the androstane analogs does not materially modify the physiological potency, but in the estrogenic and progestational types the activity is either decreased markedly or destroyed completely. Synthetic D-homoequilenins prepared by Bachmann,[438] however, have about the same potency as the corresponding equilenins.

Ruzicka [439] has suggested that this alteration of the size of ring D in the steroid nucleus is a type of change that can occur in any of the rings. To describe the potential new members of the group, he has proposed the prefix "homo" with a suitable letter to indicate in which ring alteration in size has occurred. Thus, the compounds in which ring D is six-membered will be known as D-homosteroids, while those,
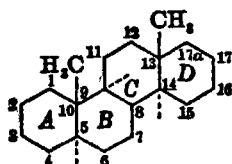
XI. D-Homoandrostane   XII. A-Homoandrostane   XIII. A-Nor-D-homoandrostane
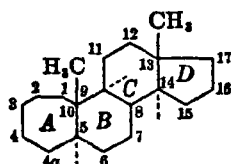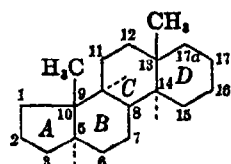
as yet unrealized, in which expansion of ring A, for example, has occurred, will be described as A-homosteroids. Where a new carbon atom is introduced, it will be designated by a number and the letter "a," for example, 17a and 4a, as shown in D-homoandrostane (X), and A-homoandrostane (XI). Where contraction of ring size takes place, Ruzicka proposes to drop the number of the carbon atom eliminated, and illustrates this practice by the hypothetical A-nor-D-homoandrostane (XII).

BIOGENESIS OF THE STEROIDS

Several possible mechanisms for the biochemical formation of the steroids have been suggested, but none has been established. In the earlier speculations, squalene, isoprene, and carbohydrates were considered precursors of the sterols. In later hypotheses, the existence of a mechanism for the formation of the sterols was assumed, and schemes were devised to show how these were degraded *in vivo* to the bile acids or to the sex hormones. Among these are the speculations of Ruzicka [440] and of Butenandt,[441] in which Δ^5-pregnenolone and dehydroandrosterone are regarded as biochemical degradation products of the sterols. With these two compounds as intermediates, oxidation and reduction or demethylation can conceivably lead to the sex hormones and to

[440] Ruzicka, *Helv. Chim. Acta*, **19**, E89 (1936).
[441] Butenandt, *Naturwissenschaften*, **24**, 529 (1936).

related compounds. A more flexible theory has been advocated by Reichstein,[642] who has suggested that the three-carbon carbohydrates, such as dihydroxyacetone or glyceric aldehyde, may condense as indicated in I. A later suggestion, advanced by Marker[643] to explain the formation only of the steroid hormones, visualizes $\Delta^{4,8(9)}$-pregnadiene-17,21-diol-3,11,20-trione (II) as the common precursor of the estrogens, the pregnanes, and the androgens; this suggestion may be regarded as a special case of Reichstein's theory.
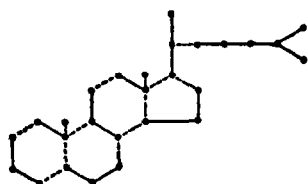
I. Schematic biochemical formation of steroids from 3-carbon sugars (Reichstein)
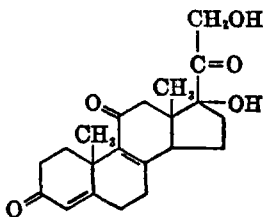
II. $\Delta^{4,8(9)}$-Pregnadiene-17,21-diol-3,11,20-trione

Although the outcome of biochemical study of the origin and interconversion of the steroids has been disappointing,[644] some positive results have been obtained from the study of the action of the microorganisms on the sex hormones.[645] Yeast, under anaerobic conditions, reduces carbonyl groups at C_3 and C_{17} in the androstane group but does not attack a C_3 carbonyl group in conjugation with a C_4 ethylenic linkage or the double bond itself. For example, Δ^4-androstene-3,20-dione (III) is reduced by yeast to testosterone (IV).[646] On the other hand, if the C_3 carbonyl group is conjugated with a double bond at $C_1 : C_2$, both the carbonyl group and the double bond are reduced.[647] In all cases, an α-configuration of the hydroxyl groups is formed; this appears to be true also in the biochemical reduction of estrone[648] and of progesterone[649] with yeast. Under aerobic conditions, the *Corynebacterium* from certain yeasts brings about the reverse of this change, and testosterone, for example, is dehydrogenated to Δ^4-androstene-3,20-dione.[650] *Bacillus putrificus* (?), isolated from putrefying steers' testes, is another

[642] Reichstein, *Helv. Chim. Acta*, **20**, 978 (1937).

[643] Marker, *J. Am. Chem. Soc.*, **60**, 1725 (1938).

[644] *Inter al.*, Anchel and Schoenheimer, *J. Biol. Chem.*, **125**, 23 (1938); Tukamoto, *Z. physiol. Chem.*, **260**, 210 (1939); Dirscherl and Trant, *ibid.*, **262**, 61 (1939).

[645] Review: Fischer, *Angew. Chem.*, **53**, 461 (1940).

[646] Mamoli and Vercellone, *Ber.*, **70**, 470 (1937).

[647] Butenandt, Dannenberg, and Suranyi, *Ber.*, **73**, 818 (1940).

[648] Mamoli, *Ber.*, **71**, 2696 (1938); Wettstein, *Helv. Chim. Acta*, **22**, 250 (1939).

[649] Mamoli, Koch, and Teschen, *Z. physiol. Chem.*, **261**, 287 (1939).

[650] Mamoli, Koch, and Teschen, *Naturwissenschaften*, **27**, 319 (1939); *cf.* Mamoli *Ber.*, **72**, 1863 (1939).

microörganism that has been studied. In sterile yeast water, *B. pu-trificus* reduces both Δ^4-unsaturation and a C_{17} carbonyl group without affecting a C_3 carbonyl group; but in a sterile yeast suspension the organism acts also on the C_3 carbonyl group.[645] This is shown in the conversion of Δ^4-androstene-3,20-dione (III) through the stage of 3,20-etiocholanedione (V) to etiocholan-17(α)-ol-3-one (VI), and of testo-sterone (IV) to etiocholan-17(α)-ol-3-one (VI) by *B. putrificus* in sterile yeast water; and in the production of 3(α),17(α)-etiocholanediol (VII) by the same organism in sterile yeast suspension.[651] Although these bio-

III. Δ^4-Androstene-3,20-dione   IV. Testosterone
V. 3,20-Etiocholanedione   VI. Etiocholan-17(α)-ol-3-one   VII. 3(α),17(α)-Etiocholanediol
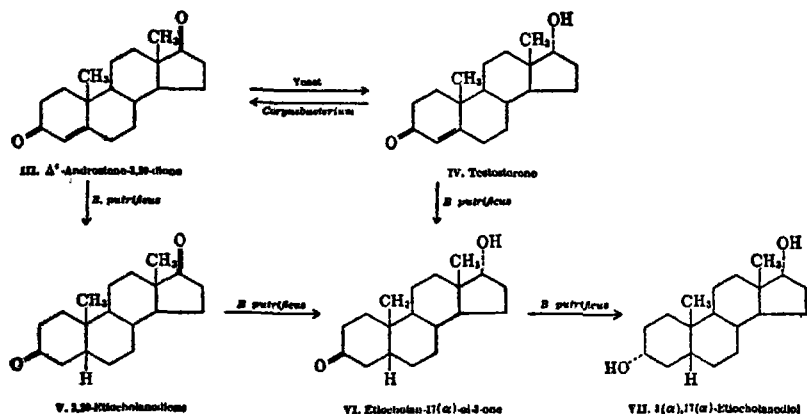
chemical reductions lead to a *cis* configuration at C_5, under some con-ditions[652] equal quantities of the corresponding *allo* structures are pro-duced.

GENERAL REFERENCES

During the last decade many monographs and reviews dealing with the chemistry of the steroids have appeared. In addition, more than 250 U. S. patents have been granted on the preparation and uses of steroids and their derivatives. Most of the patents are assigned by the Patent Office to Class 260–397. The preparation and uses of compounds with vitamin D, and with hormonal activity, have been patented more than any other phase of the field.

Various reviews of the field have appeared in Abderhalden's "Bio-chemisches Handlexikon" and "Handbuch der biologischen Arbeits-methoden" and in Oppenheimer's "Handbuch der Biochemie." Many of these treatments have been written from the older point of view and

[651] and Mamoli, *Ber.*, **71**, 156 (1938); Ercoli, *Ber.*, **71**, 650 (1938); Schramm and M., **71**, 1322, 2083 (1938).
[652] amm and Mamoli, *Ber.*, **71**, 2608 (1938); Ercoli, *Ber.*, **72**, 190 (1939).

are of value largely for experimental details and physical constants. The more important modern monographs and reviews are listed below:

Monographs

SHIMIZU, "Über die Chemie und Physiologie der Gallensäuren," Muramoto, Okayama (1935), 388 pages.

LETTRÉ and INHOFFEN, "Über Sterine, Gallensäuren und verwandte Naturstoffe," Enke, Stuttgart (1936), 320 pages.

FIESER, "The Chemistry of Natural Products Related to Phenanthrene" (A. C. S. monograph 70), Reinhold Publishing Corp., New York (1937), 2nd ed., 456 pages.

FRIEDMANN, "Sterols and Related Compounds," Chemical Publishing Co., New York (1937), 100 pages.

SOBOTKA, "The Chemistry of the Sterids," Williams and Wilkins, Baltimore (1938), 634 pages.

Reviews

Structure: WINDAUS, Z. physiol. Chem., **213**, 147 (1932); HEILBRON, SIMPSON, and SPRING, J. Chem. Soc., 626 (1933).

Sterols: BILLS, Physiol. Reviews, **15**, 1 (1935); HEILBRON and SPRING, Fortschritte der Chemie organ. Naturstoffe, Springer, Vienna (1938), **1**, 53; HEILBRON and JONES, Ann. Rev. Biochem., **9**, 135 (1940).

Bile Acids: SOBOTKA, Chem. Rev., **15**, 311 (1934).

Cardiac Aglucons and Toad Poisons: ELDERFIELD, Chem. Rev., **17**, 187 (1935); TSCHESCHE, Ergeb. Physiol., **38**, 31 (1936); STOLL, "The Cardiac Glycosides," The Pharmaceutical Press, London (1937); Schoppee, Ann. Rev. Biochem., **11**, 123 (1942).

Digitalis Saponins: TSCHESCHE, Ergeb. Physiol., **38**, 65 (1936); Schoppee, Ann. Rev. Biochem., **11**, 103 (1942).

Estrogenic Hormones: STORMER and WESTPHAL, Ergeb. Physiol., **35**, 315 (1933); MARRIAN, Ergeb. Vitamin-Hormonforsch., **1**, 419 (1938); DOISY, Chapter XIII, in Allen, "Sex and Internal Secretions," Williams and Wilkins Co., 2nd ed., Baltimore (1939).

Corpus Luteum Hormone: WESTPHAL, Ergeb. Physiol., **37**, 273 (1935); ALLEN, Chapter XV, in Allen, "Sex and Internal Secretions," Williams and Wilkins Co., 2nd ed., Baltimore (1939); WESTPHAL, Naturwissenschaften, **28**, 445 (1940).

Androgenic Hormones: GOLDBERG, Ergeb. Vitamin-Hormonforsch., **1**, 371 (1938); KOCH, Chapter XII, in Allen, "Sex and Internal Secretions," Williams and Wilkins Co., 2nd ed., Baltimore (1939).

Adrenal Hormones: REICHSTEIN, Ergeb. Vitamin-Hormonforsch., **1**, 334 (1938); MIESCHER, Angew. Chem., **51**, 551 (1938).

CHAPTER 20

CARBOHYDRATES I

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CONTENTS

INTRODUCTION

The research field concerned with the development of the fundamental organic chemistry of the carbohydrates has been a very active one and is still the object of a large amount of research. The great organic chemist Emil Fischer made his first mark here, and many others have been attracted by this fascinating group of substances. The carbohydrates may be classified from the standpoint of their hydrolytic products into monosaccharides, oligosaccharides, and polysaccharides; the last two groups produce monosaccharides on hydrolysis; the molecular complexity of the oligosaccharides has been ascertained, but that of the polysaccharides is still not known with absolute certainty. The monosaccharides are polyhydroxy aldehydes and ketones that reduce mild alkaline oxidizing agents, such as Fehling's solution. They may be further classified according to the length of their carbon chain and according to the nature of their carbonyl function; thus there are aldopentoses, aldohexoses, ketohexoses, etc. The monosaccharides are colorless crystalline solids and possess a sweet taste.

The central compound of the carbohydrates is d-glucose, and any development of the subject of carbohydrate chemistry from a research problem standpoint must revolve about this substance. d-Glucose is a monosaccharide classified as an aldohexose. It is the most readily available of the monosaccharides and the most important one from the standpoint of animal metabolism. Since all the monosaccharides are polyhydroxy ketones or aldehydes, it follows that knowledge gained by an investigation of the d-glucose molecule can generally be extended to its many relatives. This extension is not always easily accomplished, and of course significant and interesting differences in reactivity are exhibited by the various other monosaccharides.

The development of our present conception of the structure of d-glucose represents a fascinating chapter in the evolution of a chemical formula. As new experimental evidence was obtained, previous ideas had to be revised in the sense that the old views were not wrong but were incomplete. Thus the formula of d-glucose stands today as a representation of one of the most thoroughly investigated substances in the entire field of organic chemistry.

The original sweetening agent native to Europe was the sugar mixture known as honey. Alexander the Great is credited with introducing cane sugar into Europe from the Orient, and purified cane sugar or sucrose was undoubtedly the first crystalline sugar known. Ironically, of all the sugars, the formula of this substance has proved to be the most difficult to unravel. Since the sugar cane could be grown only in the

tropics, a search was made in Europe for a native plant substitute amenable to field cultivation, and this culminated successfully in the beet-sugar industry, established by Achard [1] (1798). In the course of this search, Marggraf [2] (1747) had crystallized a substance which he recognized as being different from cane sugar and which he termed "eine Art Zucker." This was the substance which is now called glucose. Sucrose crystallizes very readily. Glucose, on the contrary, is a difficult substance to crystallize, and it is only within the past few years that crystalline glucose has been produced commercially at a low cost.

The writings of Marggraf do not constitute the first record of crystalline glucose. This had been prepared previously from a variety of sources, but especially from grapes, and was known to the ancient Persians and Arabians. Reference to this grape sugar can be found in the old Moorish records [3] (1150) and in the writings of the alchemists and early pharmacists.

Elementary analysis of glucose produced the empirical formula CH_2O. This formula represents the origin of the French term *hydrate de carbone*, which was modified in the German to *Kohlenhydrat* and the latter translated into English as carbohydrate. A molecular-weight determination showed that the true formula was $C_6H_{12}O_6$. This result was not obtained until 1888 (Tollens and Mayer),[4] as no method was available for determining its molecular weight until the appearance of the work of Raoult [5] (1880) and the Beckmann apparatus [6] (1888).

That glucose contained five acetylatable hydroxyl groups was proved definitely by Franchimont [7] (1879 and 1892), who obtained its first crystalline pentaacetate. This, together with its reducing properties, gave the formula $C_5H_7(OH)_5$—CO. It is necessary to emphasize here that real progress in the chemistry of the sugars can result only on the basis of pure crystalline derivatives. The failure to recognize this premise adequately has led to many grievous errors and much confusion.

Kiliani [8] then improved the procedure of Schützenberger [9] (1881) for forming the cyanohydrin of glucose, hydrolyzed this to the acid, reduced

[1] Achard, "Die europäische Zuckerfabrikation aus Runkelrüben," Hinrichs, Leipzig (1809); cf., von Lippmann, "Geschichte des Zuckers," 2nd ed., Springer, Berlin (1929), pp. 698, 700, 701.

[2] Marggraf, Ber. Berliner Akad., V, 79 (1749); cf., von Lippmann, "Geschichte des Zuckers," 2nd ed., Springer, Berlin (1929), p. 683.

[3] Ibn Al-Awam, II, 398, 400; cf., von Lippmann, "Geschichte des Zuckers," 2nd ed., Springer, Berlin (1929), p. 682.

[4] Tollens and Mayer, Ber., 21, 1566 (1888).

[5] Raoult, Ann. chim. phys., [5] 20, 217 (1880).

[6] Beckmann, Z. physik. Chem., 2, 638 (1888).

[7] Franchimont, Ber., 12, 1940 (1879); Rec. trav. chim., 11, 106 (1892).

[8] Kiliani, Ber., 19, 767 (1886).

[9] Schützenberger, Bull. soc. chim., [2] 36, 144 (1881).

the latter with hydriodic acid, and obtained *n*-heptoic acid (1886). This proved that an aldehyde group was present and that the six carbon atoms of glucose were arranged in a straight or normal chain, thus indicating the formula $CH_2OH—(CHOH)_4—CHO$. Kiliani [10] (1886) also applied the above procedure to fructose, which was an isomer of glucose isolated by Dubrunfaut [11] (1856) from invert sugar through its property of forming an insoluble compound with lime. Kiliani arrived at the formula $CH_2OH—(CHOH)_3—CO—CH_2OH$ for this substance and thus demonstrated the existence of a polyhydroxy ketone or ketose.

CONFIGURATIONAL ISOMERISM OF THE MONOSACCHARIDES

It is to be noted that the Kiliani formula for glucose contained four asymmetric carbon atoms and according to the van't Hoff [12]-Le Bel [13] (1874) theory, which was at that time quite new, there were 2^4 or sixteen possible isomers for this compound. Examples of such isomers had begun to appear, as for instance, the isolation of galactose in 1856 by Louis Pasteur.[14] The simple sugars that were isolated from natural sources and found to be different from glucose were considered to be isomeric with it and assigned the same formula. One of these supposed isomers was arabinose, and on closer investigation of this substance Kiliani [15] (1886) was surprised to find that it contained only five carbon atoms. Thus was established the first member of the group of sugars known as the aldopentoses.. The problem of determining the spatial configuration of all these isomers appeared quite hopeless. To add to the difficulty, the substances and their derivatives were difficult to crystallize, and they were also very sensitive to heat and to strong reagents. The time was thus ripe for a genius to arise who would possess the ability and industry to bring order out of chaos, and this genius was Emil Fischer.

Emil Fischer was at the time concerned with the apparently unrelated problem of preparing substitution products of hydrazine, among which was phenylhydrazine. He noted that the latter compound served quite well for preparing derivatives of aldehydes and ketones and he tried its action with the sugars [16] (1884). Certainly he must have been astonished to find that crystalline substances were formed with surprising ease. However, these did not analyze correctly for phenylhydrazones, two

[10] Kiliani, *Ber.*, **19**, 221 (1886).

[11] Dubrunfaut, *Compt. rend.*, **42**, 901 (1856).

[12] van't Hoff, "Sur les formules de structure dans l'espace," *Arch. néerland. sci.* (1874).

[13] Le Bel, *Bull. soc. chim.*, [2] **22**, 337 (1874).

[14] Pasteur, *Compt. rend.*, **42**, 347 (1856).

[15] Kiliani, *Ber.*, **19**, 3029 (1886); **20**, 339 (1887).

[16] Fischer, *Ber.*, **17**, 579 (1884).

phenylhydrazine residues having entered the molecule. On further investigation [17] (1887) he found that oxidation on the carbon atom adjacent to the aldehyde group had taken place with the formation of aniline, ammonia, and the substance he termed the phenylosazone. He was able to find the true phenylhydrazone as the expected intermediate, this substance being in most cases too soluble for ready separation. He noted [18] (1884) that the phenylosazone obtained from glucose was identical with that obtained from fructose, and a start was made on the problem of solving the spatial configuration of the sugars.

$$
\begin{array}{ccc}
\text{HC}=\text{O} & & \text{HC}=\text{NNHC}_6\text{H}_5 \\
| & \xrightarrow{\ \text{NH}_2\text{NHC}_6\text{H}_5\ } & | \\
(\text{CHOH})_4 & & (\text{CHOH})_4 + \text{H}_2\text{O} \xrightarrow{\ \text{NH}_2\text{NHC}_6\text{H}_5\ } \\
| & & | \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH} \\
\text{Glucose} & & \text{Glucose phenylhydrazone}
\end{array}
$$

$$
\begin{array}{ccc}
\text{HC}=\text{NNHC}_6\text{H}_5 & & \text{HC}=\text{NNHC}_6\text{H}_5 \\
| & & | \\
\text{C}=\text{O} & \xrightarrow{\ \text{NH}_2\text{NHC}_6\text{H}_5\ } & \text{C}=\text{NNHC}_6\text{H}_5 \\
| & & | \\
(\text{CHOH})_3 + \text{NH}_3 + \text{C}_6\text{H}_5\text{NH}_2 & & (\text{CHOH})_3 + \text{H}_2\text{O} \\
| & & | \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH} \\
& & \text{Glucose phenylosazone}
\end{array}
$$

$$
\begin{array}{cc}
\text{CH}_2\text{OH} & \text{HC}=\text{NNHC}_6\text{H}_5 \\
| & | \\
\text{C}=\text{O} \xrightarrow{\ 3\text{NH}_2\text{NHC}_6\text{H}_5\ } & \text{C}=\text{NNHC}_6\text{H}_5 \\
| & | \\
(\text{CHOH})_3 & (\text{CHOH})_3 + 2\text{H}_2\text{O} + \text{NH}_3 + \text{C}_6\text{H}_5\text{NH}_2 \\
| & | \\
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} \\
\text{Fructose} & \text{Glucose phenylosazone}
\end{array}
$$
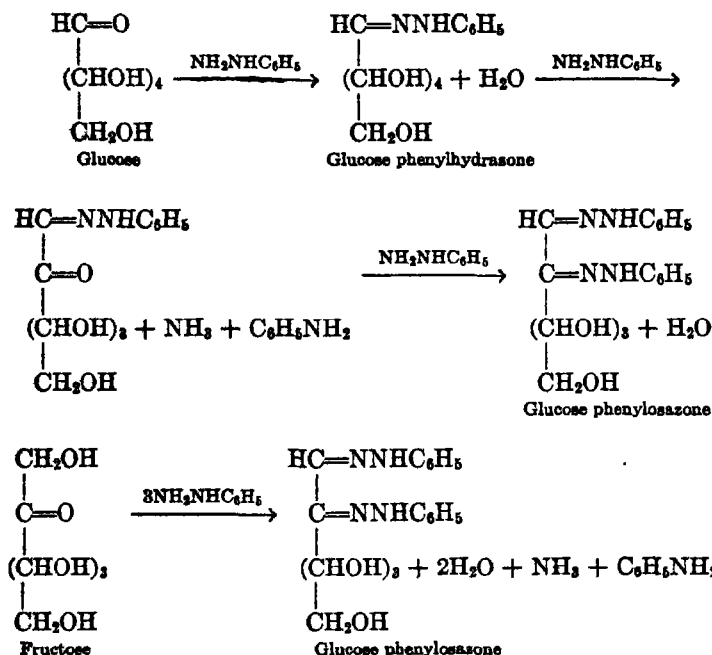
Sugars producing the same osazone thus had identical structures on all but the first two carbon atoms. The aldohexose d-mannose also yielded d-glucose phenylosazone and accordingly differed from glucose only in the configuration of the carbon atom adjacent to the aldehyde group. Aldoses bearing such a relationship are now termed epimers (p. 247), a name suggested by Votoček [19] (1911).

The three fundamental procedures used by Fischer (1884–1894) in his great feat of elucidating the configuration of the sugars were osazone

[17] Fischer, *Ber.*, **20**, 821 (1887).
[18] Fischer, *Ber.*, **17**, 579 (1884).
[19] Votoček, *Ber.*, **44**, 362 (1911).

formation, oxidation to *meso* acids or reduction to *meso* alcohols, and the methods for building up or degrading the members of the sugar series. The formation of the *meso* or optically inactive and unresolvable compounds indicated the symmetrical or internally compensated structures (p. 232). The reactions involved in the Fischer procedures will be discussed in some detail before their application to the solution of configurational problems is illustrated. These procedures constitute fundamental reactions of the aldoses and have been used for the synthesis of the various members of the aldose series.

These aldose synthetic methods employ a naturally occurring sugar as the starting point and transform this into other aldoses by various procedures. Fischer did attain a complete glucose synthesis by developing the experiments of Butlerow [20] (1861), who had noted that formaldehyde and alkali produce sugars. This interesting reaction was further investigated by Fischer. He obtained a low yield of racemic glucose phenylosazone from the mixture [21] (1889) and then skillfully completed the difficult steps from racemic glucose phenylosazone to *d*-glucose [22] (1890). Since formaldehyde can be synthesized from its elements, a complete glucose synthesis was thus accomplished.

The aldose oxidation techniques were initiated mainly by Kiliani. On hypobromite oxidation, the aldoses produce the corresponding aldonic acids, a reaction that was greatly simplified by Isbell [23] (1931), who produced the hypobromite ion by continuous electrolysis in a sugar solution containing a small amount of bromide ion, hydrobromic acid being the reduction product and this in turn being electrolyzed to form again hypobromite ion. Nitric acid oxidizes an aldose to a dibasic hydroxy acid, the aldehyde group and the terminal primary alcohol being the points of attack.

$$CHO \atop (CHOH)_4 \atop CH_2OH$$
d-Glucose

OBr' / HNO_3

$$CO_2H \atop (CHOH)_4 \atop CH_2OH$$
d-Gluconic acid

$$CO_2H \atop (CHOH)_4 \atop CO_2H$$
d-Glucosaccharic acid
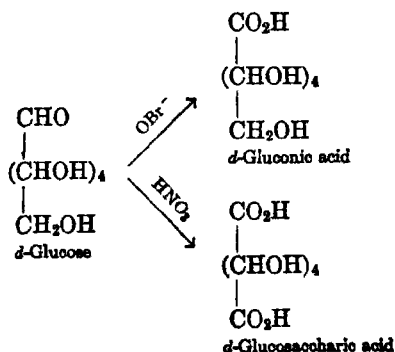
[20] Butlerow, *Ann.*, **120**, 295 (1861).
[21] Fischer and Passmore, *Ber.*, **22**, 359 (1889).
[22] Fischer, *Ber.*, **23**, 370, 799 (1890).
[23] Isbell and Frush, *Bur. Standards J. Research*, **6**, 1145 (1931).

The aldoses may also be reduced to the corresponding sugar alcohols by various reducing agents, such as sodium amalgam. The reaction proceeds very slowly with chemical reducing agents, and at present the most promising procedures are those involving electrolytic methods or high-pressure catalysis [24] (Ipatieff, 1912). The electrolytic reduction of glucose is a commercial process in this country.[25]

$$
\begin{array}{ccc}
\text{CHO} & & \text{CH}_2\text{OH} \\
| & & | \\
(\text{CHOH})_4 & \xrightarrow{[\text{H}]} & (\text{CHOH})_4 \\
| & & | \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH} \\
d\text{-Glucose} & & (\text{dextro})\text{-Sorbitol}
\end{array}
$$
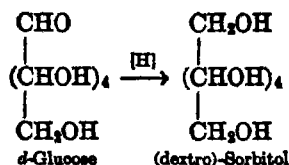
The cyanohydrin procedure for adding a carbon atom to an aldose had been developed by Kiliani, as previously noted. He had always isolated only one product from the reaction. For example, the cyanohydrin reaction when applied by him to arabinose [26] (1886 and 1887) produced the lactone of a new hexonic acid, later found to be l-mannonic. On repetition of this work Fischer [27] (1890) found the two products required by theory, one of which was the enantiomorph of d-gluconic acid. The two products can be predicted because a new asymmetric carbon atom is formed. They will be formed in unequal amounts as a new asymmetric center has been added to a molecule already asymmetric (p. 230).

The aldonic acids undergo lactonization with ease, and this is the form in which they are generally obtained although a number of the

$$
\begin{array}{ccccc}
& & \text{CN} & & \text{CO}_2\text{H} \\
& & | & & | \\
& & \text{HCOH} & \xrightarrow{\text{HOH}} & \text{HCOH} \\
& & | & & | \\
& & (\text{CHOH})_2 & & (\text{CHOH})_2 \\
\text{CHO} & \nearrow^{\text{HCN}} & | & & | \\
| & & \text{CH}_2\text{OH} & & \text{CH}_2\text{OH} \\
(\text{CHOH})_2 & & & & \text{l-Mannonic acid} \\
| & \searrow_{\text{HCN}} & \text{CN} & & \text{CO}_2\text{H} \\
\text{CH}_2\text{OH} & & | & & | \\
\text{l-Arabinose} & & \text{HOCH} & \xrightarrow{\text{HOH}} & \text{HOCH} \\
& & | & & | \\
& & (\text{CHOH})_2 & & (\text{CHOH})_2 \\
& & | & & | \\
& & \text{CH}_2\text{OH} & & \text{CH}_2\text{OH} \\
& & & & \text{l-Gluconic acid}
\end{array}
$$
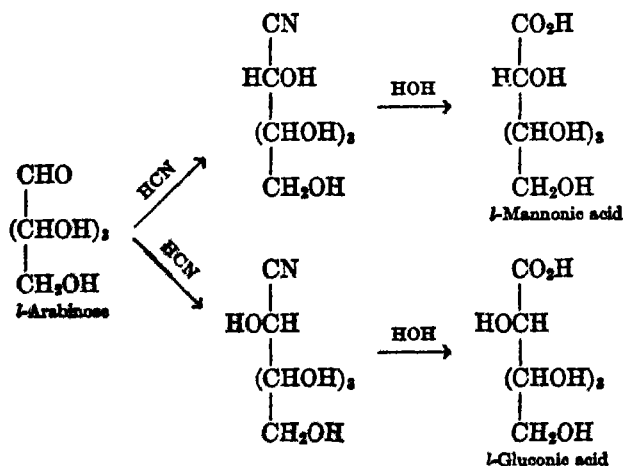
[24] Ipatieff, *Bull. soc. chim.*, [4] **14**, 552 (1913); *Ber.*, **45**, 3218 (1912).
[25] Creighton, *Trans. Electrochem. Soc.*, **75**, 289 (1939).
[26] Kiliani, *Ber.*, **19**, 3029 (1886); **20**, 339 (1887).
[27] Fischer, *Ber.*, **23**, 2611 (1890).

free acids have been prepared by crystallization from solvents in which lactone formation is hindered (Rehorst,[29] 1928; Isbell and Frush,[29] 1933). Kiliani [30] (1887) made the remarkable observation that the double lactone of mannosaccharic acid could be reduced to the sugar alcohol mannitol with sodium amalgam. Fischer [31] (1889) found that this reaction was general for the sugar lactones and made an important extension by finding that, when the procedure was carried out under slightly acidic conditions, the reduction stopped at the aldose stage. In this way Fischer obtained the epimeric higher carbon sugars of d-glucose, calling the one that crystallized α-glucoheptose and the one that did not β-glucoheptose. By repeating the process by which glucose was changed to a heptose, Fischer [32] (1892) was able to make a gluconononose, and Philippe [33] (1912) carried this to the decose stage.

The naming of the higher sugars according to their order of isolation has led to a confused state of nomenclature. For those whose complete stereochemical structure has been elucidated, Hudson [34] (1938) has suggested an overlapping type of nomenclature. Thus the name d-gluco-d-guloheptose is assigned to d-[α-glucoheptose] and indicates that this heptose is built up from d-glucose but that the configuration (p. 1543) downward from the aldehydic carbon atom is that of d-gulose.
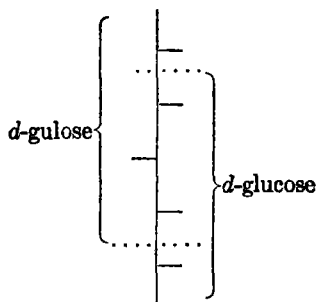
In addition to the above-described synthesis of epimers through the cyanohydrin reaction and reduction of the resultant lactones, Fischer also employed the aldonic acids directly for epimer synthesis. This important general reaction resulted from his discovery [35] (1890) that an aldonic acid is converted in part into its epimeric acid on heating its solution with a mild base, such as pyridine. The two acids could

[29] Rehorst, Ber., 61, 163 (1928); 63, 2279 (1930).

[29] Isbell and Frush, Bur. Standards J. Research, 11, 649 (1933).

[30] Kiliani, Ber., 20, 2714 (1887).

[31] Fischer, Ber., 22, 2204 (1889).

[32] Fischer, Ann., 270, 64 (1892).

[33] Philippe, Ann. chim. phys., [8] 26, 289 (1912).

[34] Hudson, J. Am. Chem. Soc., 60, 1537 (1938).

[35] Fischer, Ber., 23, 799 (1890).

then be separated and the desired epimeric lactone reduced to the aldose. A later synthesis of epimers employing the hydroxylation of the glycals is described in the succeeding chapter (p. 1628).

$$
\begin{array}{cccc}
\text{CO}_2\text{H} & \text{CO}_2\text{H} & \text{CO} & \text{CHO} \\
| & | & | \backslash & | \\
\text{HCOH} & \text{HOCH} & \text{HOCH} & \text{HOCH} \\
| & \xrightleftharpoons{\text{Pyridine}} | & \rightarrow | & \rightarrow | \\
\text{HOCH} & \text{HOCH} & \text{HOCH} & \text{HOCH} \\
| & | & | & | \\
\text{HCOH} & \text{HCOH} & \text{HCO}\!\!\!-\!\!\rfloor & \text{HCOH} \\
| & | & | & | \\
\text{HCOH} & \text{HCOH} & \text{HCOH} & \text{HCOH} \\
| & | & | & | \\
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} \\
\textit{d-}\text{Gluconic acid} & \textit{d-}\text{Mannonic acid} & \textit{d-}\text{Mannonolactone} & \textit{d-}\text{Mannose}
\end{array}
$$
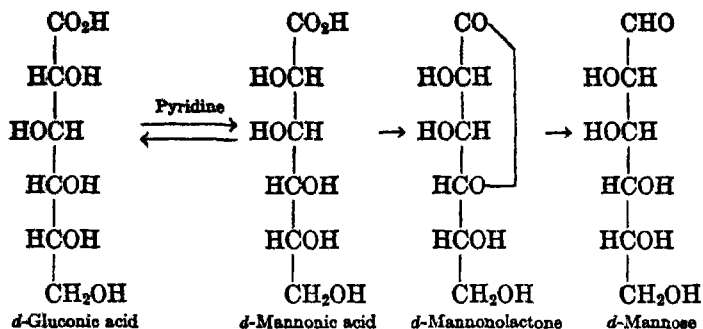
The sugar degradation methods extended and confirmed the facts obtained by the cyanohydrin reactions. The first method used was that developed by Wohl. Wohl [36] (1893) acetylated d-glucose oxime and obtained gluconic acid nitrile pentaacetate. Treatment of this substance with an ammoniacal solution of silver oxide produced the diacetamido compound of arabinose, and this on acid hydrolysis yielded d-arabinose or the enantiomorph of the common naturally occurring pentose.

$$
\begin{array}{ccc}
\text{CHO} & \text{HC}\!\!=\!\!\text{NOH} & \text{CN} \\
| & | & | \\
(\text{CHOH})_4 \xrightarrow{\text{H}_2\text{NOH}} & (\text{CHOH})_4 \xrightarrow[\text{NaOAc}]{\text{Ac}_2\text{O}} & \text{HCOAc} \xrightarrow{\text{Ag(NH}_3)_2\text{OH}} \\
| & | & | \\
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} & (\text{CHOAc})_3 \\
\textit{d-}\text{Glucose} & & | \\
& & \text{CH}_2\text{OAc}
\end{array}
$$

$$
\begin{array}{ccc}
\text{HC(NHCOCH}_3)_2 & & \text{CHO} \\
| & & | \\
(\text{CHOH})_3 & \xrightarrow[(\text{H}_2\text{SO}_4)]{\text{HOH}} & (\text{CHOH})_3 \\
| & & | \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH} \\
& & \textit{d-}\text{Arabinose}
\end{array}
$$
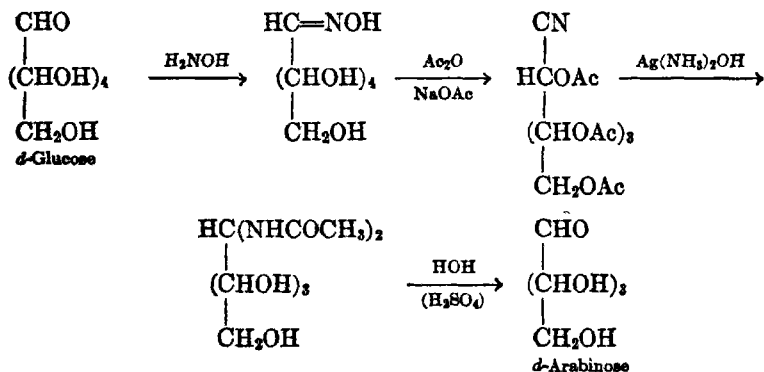
This procedure gives fair results with most of the monosaccharides, and Zemplén later extended it to the disaccharides by replacing the ammoniacal silver solution with sodium ethoxide. In this manner Zemplén [37] (1926) degraded the disaccharides cellobiose and lactose by one carbon atom. The next degradation method was developed by Ruff [38] (1898) and is the one that has been the more successful for pre-

[36] Wohl, *Ber.*, **26**, 730 (1893).

[37] Zemplén, *Ber.*, **59**, 1254, 2402 (1926).

[38] Ruff, *Ber.*, **31**, 1573 (1898); *cf.* Hockett and Hudson, *J. Am. Chem. Soc.*, **56**, 1632 (1934).

parative purposes. It is a modification of the Fenton [39] (1893) reaction. The calcium salt of the sugar acid is treated with hydrogen peroxide in the presence of ferric ion, and degradation to the next lower aldose is effected. Weerman [40] (1918) developed a degradation method based upon the action of hypochlorite upon the amide of the sugar acid, but this extension of the old Hofmann reaction (p. 983) has not received much application.

Having described the important procedures available to Emil Fischer, their use will be illustrated by indicating how they were applied in determining the structure of the five carbon aldoses or pentoses. Eight (2^3) active forms are theoretically possible, and the d- and l-forms of arabinose, xylose, lyxose, and ribose were the compounds eventually made known through the labors of the Fischer school and others. The possible configurations are the four given (p. 1542) and their enantiomorphs.

Since arabinose and ribose give the same osazone and are consequently epimeric, they must be either (1) and (2) or (3) and (4). Lyxose and xylose likewise give identical osazones. Arabinose on nitric acid oxidation gives an optically active trihydroxyglutaric acid, hence arabinose can have only the configuration (2) or (4). Furthermore, arabinose with hydrogen cyanide and subsequent hydrolysis and oxidation gives two *active* dicarboxylic acids, and must therefore have the structure (2), as one of the acids derived in this way from (4) would be optically inactive by internal compensation. If (2) represents d-arabinose, then its enantiomorph represents l-arabinose, and (1) must represent d-ribose. Xylose yields an inactive trihydroxyglutaric acid when oxidized and must then be represented by configuration (3), and hence lyxose is (4). Since l-arabinose, when treated with hydrogen cyanide and the product hydrolyzed and reduced, yields a mixture of l-glucose and l-mannose, it follows that in these last two the spatial arrangement of their carbon atoms three to five, inclusive, is identical with that of l-arabinose. This is confirmed by the fact that d-glucose produces d-arabinose on degradation by one carbon atom and thus d-arabinose is configurationally related to dextrorotatory or d-glucose.

In the above reasoning no indication is given regarding which of the enantiomorphs of each pentose corresponds to the assigned numbers. Fischer used d-glucose and the tartaric acids as his reference compounds, and this led to ambiguities in the gulose-idose aldohexose series which arose from Fischer's naming the gulose obtained from d-glucose as the d-form. Rosanoff [41] (1906) has shown that in order to obtain a

[39] Fenton, *Proc. Chem. Soc.*, **9**, 113 (1893).
[40] Weerman, *Rec. trav. chim.*, **37**, 16 (1918).
[41] Rosanoff, *J. Am. Chem. Soc.*, **28**, 114 (1906).

solution of this problem which is free from ambiguities it is necessary to choose the aldose containing only one asymmetric center as the ultimate

$$
\begin{array}{cccc}
\text{CHO} & \text{CHO} & \text{CHO} & \text{CHO} \\
\text{HCOH} & \text{HOCH} & \text{HCOH} & \text{HOCH} \\
\text{HCOH} & \text{HCOH} & \text{HOCH} & \text{HOCH} \\
\text{HCOH} & \text{HCOH} & \text{HCOH} & \text{HCOH} \\
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} \\
(1) & (2) & (3) & (4) \\
d\text{-Ribose} & d\text{-Arabinose} & d\text{-Xylose} & d\text{-Lyxose}
\end{array}
$$

HC=NNHC$_6$H$_5$
C=NNHC$_6$H$_5$
HCOH
HCOH
CH$_2$OH

HC=NNHC$_6$H$_5$
C=NNHC$_6$H$_5$
HOCH
HCOH
CH$_2$OH

(O)

$$
\begin{array}{cccc}
\text{CO}_2\text{H} & \text{CO}_2\text{H} & \text{CO}_2\text{H} & \text{CO}_2\text{H} \\
\text{HCOH} & \text{HOCH} & \text{HCOH} & \text{HOCH} \\
\text{HCOH} & \text{HCOH} & \text{HOCH} & \text{HOCH} \\
\text{HCOH} & \text{HCOH} & \text{HCOH} & \text{HCOH} \\
\text{CO}_2\text{H} & \text{CO}_2\text{H} & \text{CO}_2\text{H} & \text{CO}_2\text{H} \\
\text{(Optically inactive)} & & \text{(Optically inactive)} &
\end{array}
$$

HCN
+
HOH

$$
\begin{array}{cccccc}
\text{CHO} & \text{CO} & \text{CO}_2\text{H} & \text{CO}_2\text{H} & \text{CO} & \text{CHO} \\
\text{HCOH} & \text{HCOH} & \text{HCOH} & \text{HOCH} & \text{HOCH} & \text{HOCH} \\
\text{HOCH} & \text{HOCH} & \text{HOCH} & \text{HOCH} & \text{HOCH} & \text{HOCH} \\
\text{HCOH} & \text{HCO} & \text{HCOH} & \text{HCOH} & \text{HCO} & \text{HCOH} \\
\text{HCOH} & \text{HCOH} & \text{HCOH} & \text{HCOH} & \text{HCOH} & \text{HCOH} \\
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} \\
d\text{-Glucose} & & & & & d\text{-Mannose}
\end{array}
$$

(H)   +   (H)

(O)   (O)

$$
\begin{array}{cc}
\text{CO}_2\text{H} & \text{CO}_2\text{H} \\
\text{HCOH} & \text{HOCH} \\
\text{HOCH} & \text{HOCH} \\
\text{HCOH} & \text{HCOH} \\
\text{HCOH} & \text{HCOH} \\
\text{CO}_2\text{H} & \text{CO}_2\text{H}
\end{array}
$$

reference substance. This substance is glyceraldehyde, whose d-form is represented empirically below.

$$CHO$$
$$|$$
$$HCOH$$
$$|$$
$$CH_2OH$$
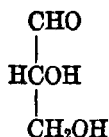
It is to be emphasized that the symbols d- and l- refer to configuration and not to sign of rotation, the conventions (dextro) and (levo) denoting the latter (p. 304). The symbol d,l- will be used to indicate a racemic form and i- to denote the inactive and non-resolvable meso form. It is well known that d(dextro)-glucose is configurationally related to (levo)-fructose, both giving the same phenylosazone. Accordingly the common form of fructose is d(levo)-fructose.

Rosanoff considered all the higher d-aldoses as being derived from d-glyceraldehyde by successive cyanohydrin syntheses. Accordingly an aldose belongs to the d-series when the hydroxyl group on the carbon directly attached to the end primary alcohol group is represented on the right in the stereochemical projection formula. The elaboration of the aldose series according to Rosanoff is given in Fig. 1. The conventional representation is that used by Rosanoff in which a horizontal line to the right indicates a hydroxyl group in that direction and the top circle represents the aldehyde group. These conventional representations may be rotated only in the plane of the paper.

According to the Rosanoff classification, the same dibasic or saccharic acid is obtained from d-glucose and l-gulose.

I   II
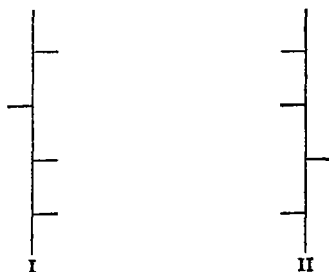
The representation of the saccharic acid from d-glucose is indicated by (I) and that from l-glucose by (II). The latter when rotated 180° in the plane of the paper is identical with (I). The configuration symbol thus loses its significance in this case, and the long-known compound prepared by Scheele [43] (1776) is then best represented merely as (dextro)-saccharic

[43] Scheele, cf. Bugge (Lockemann), "Das Buch der grossen Chemiker," Verlag Chemie, Berlin (1929), Vol. I, p. 282.

acid.   Similarly   no   configurational   assignment   can   be   given   to
sorbitol.
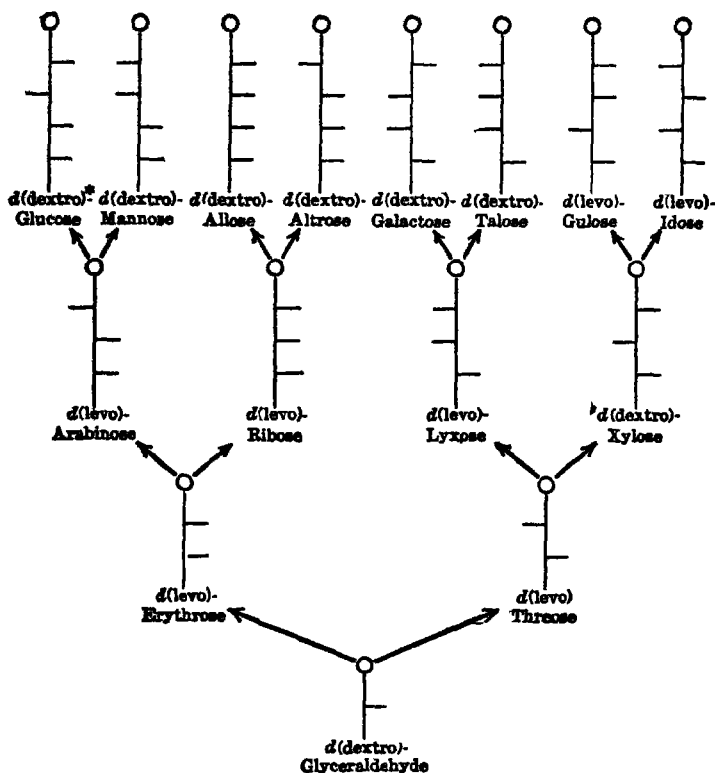
FIG. 1.—d-Series of the aldoses

Glyceraldehyde was obtainable at the time (see, however, p. 1586) only
in the racemic or d,l-form.   The problem of resolving glyceraldehyde and
adding hydrogen cyanide to the optically active forms was extremely
difficult.   The Rosanoff classification is independent of the actual ful-
fillment of this step.   However, this difficult work was finally accom-
plished by Wohl and Momber [43] (1914 and 1917), and (dextro)-glycer-
aldehyde was related to (levo)-tartaric acid.   The same (levo)-tartaric
acid had been obtained by Maquenne [44] (1901) from the oxidation of the
threose formed by the Wohl degradation of natural d-xylose, and thus
the hexose degradation methods met the cyanohydrin procedures at the

* The rotatory sign (dextro) or (levo) refers to that of the equilibrated aqueous solution
[43] Wohl and Momber, Ber., 47, 3346 (1914); 50, 455 (1917).
[44] Maquenne, Ann. chim. phys., [7] 24, 399 (1901).

tetrose stage. This leads to the designations d(dextro)-glyceraldehyde and d(levo)-tartaric acid. The assignment of the d-configuration to levorotatory tartaric acid is of course contrary to the general usage of the terms d- and l- for the tartaric acids, wherein these symbols are employed merely to denote the sign of rotation.

d(levo)-Tartaric
acid
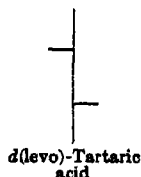
The results and significance of this work were incorporated in an important article by Wohl and K. Freudenberg [45] (1923).

RING STRUCTURE AND TAUTOMERIC FORMS

Introduction. To return again to the central compound, d-glucose, which serves as the prototype of all its relatives, it is found that the rather involved discussion just completed has advanced the Kiliani formula of

$$CH_2OH-(CHOH)_4-CHO \quad \text{to} \quad CH_2OH-\overset{H}{\underset{OH}{C}}-\overset{H}{\underset{OH}{C}}-\overset{OH}{\underset{H}{C}}-\overset{H}{\underset{OH}{C}}-CHO.$$
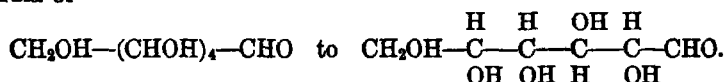
Fischer [46] (1893) next began to study further reactions of this polyhydroxy aldehyde, and one of the problems he attacked was that of its behavior, under acetal-forming conditions, with methanol and hydrogen chloride. He obtained no true acetal but instead only one methyl group entered the molecule. The product was non-reducing but showed reducing properties after acid hydrolysis. In the following year Alberda van Ekenstein [47] (1894) isolated a second isomer from the same reaction. To explain such results, Fischer adopted the ring-structure formula for these derivatives which had previously been suggested by Tollens [48] (1883) for d-glucose. At the same time Fischer correctly insisted that the facts as then known did not warrant the extension of this ring structure to d-glucose itself.

The two isomeric methanol condensation products of glucose were

[45] Wohl and Freudenberg, Ber., 56, 309 (1923).
[46] Fischer, Ber., 26, 2400 (1893).
[47] Alberda van Ekenstein, Rec. trav. chim., 13, 183 (1894)
[48] Tollens, Ber., 16, 921 (1883).

named by Fischer α-methyl-*d*-glucoside ($[\alpha]_D + 159°$) and β-methyl-*d*-glucoside ($[\alpha]_D - 34°$), and the structures assigned were:

$$
\begin{array}{ccc}
\text{H} \quad \text{OCH}_3 & & \text{CH}_3\text{O} \quad \text{H} \\
\diagdown \quad \diagup & & \diagdown \quad \diagup \\
\text{C}\!\!-\!\! & & \text{C}\!\!-\!\! \\
| & & | \\
\text{HCOH} & & \text{HCOH} \\
| & & | \\
\text{HOCH} & & \text{HOCH} \\
| & & | \\
\text{HCO}\!\!-\!\! & & \text{HCO}\!\!-\!\! \\
| & & | \\
\text{HCOH} & & \text{HCOH} \\
| & & | \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH}
\end{array}
$$
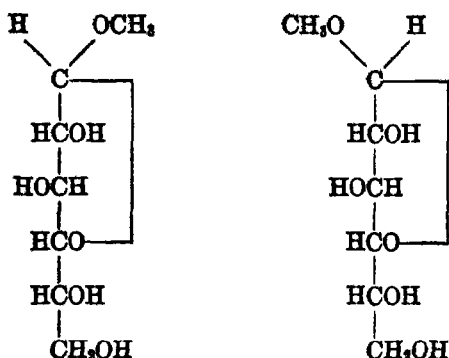
These formulas represent inner cyclic acetals, and carbon one has become asymmetric, thus accounting for the two isomers. Being compounds of the acetal type, they are stable toward alkali but are hydrolyzed by acids. Fischer placed the ring closure on the fourth carbon, by analogy with the γ-lactones. This was an arbitrary assumption which later proved to be incorrect.

This classical work of Fischer confirmed previous indications that a ring-structure assignment was needed for other derivatives of glucose. Thus, Colley [49] (1870) had suggested a ring structure to explain the reactions of a crystalline acetochloroglucose (p. 1573), and A. Michael [50] (1879) had synthesized a phenol glucoside. Skraup [51] (1889) had decided that glucose pentabenzoate contained no free aldehyde group, and finally Franchimont [52] (1879; 1892) and Erwig and Koenigs [53] (1889) had used this structure to explain the known isomeric pair of glucose pentaacetates.

Mutarotation. The extension to *d*-glucose of the ring structure clearly required by these methylglucosides involves a consideration of the phenomenon of mutarotation (p. 305). Dubrunfaut [54] (1846) observed that the optical rotation of a freshly prepared aqueous solution of glucose gradually fell to a constant value. This being about one-half of the original, he termed the phenomenon birotation. This property is possessed by all sugars, which reduce Fehling's solution, with the exception of a number of the ketoses. However, the fall to a half value

[49] Colley, *Ann. chim. phys.*, [4] **21**, 363 (1870).
[50] Michael, *Am. Chem. J.*, **1**, 305 (1879).
[51] Skraup, *Monatsh.*, **10**, 401 (1889).
[52] Franchimont, *Ber.*, **12**, 1940 (1879); *Rec. trav. chim.*, **11**, 106 (1892).
[53] Erwig and Koenigs, *Ber.*, **22**, 2207 (1889).
[54] Dubrunfaut, *Compt. rend.*, **23**, 38 (1846).

was, of course, only adventitious for glucose, and the name mutarotation as suggested by Lowry [55] (1899) is now used. Fischer, the experimentalist, did not concern himself with this rotation change other than merely to suggest that perhaps it was due to hydration.

The experimental evidence required to interpret the mutarotation of glucose was furnished by Tanret [56] (1895) when he prepared two isomeric forms of d-glucose. One of these, α-d-glucose, shows a rotation change of +113° → + 52.5°; the other, β-d-glucose, +19° → + 52.5°. They are thus mutually interconvertible into an equilibrium mixture. Tanret obtained the equilibrium mixture as peculiar mixed crystals which he thought constituted a third isomer, but other workers soon corrected this error. When a sugar crystallizes from solution, it separates almost entirely in that form which is the least soluble under the conditions, the solution equilibrium then shifting to produce more of this isomer. The preparation of a sugar in its crystalline α- and β-forms thus becomes a difficult matter as it is necessary to find conditions under which each form will crystallize. Such conditions have been realized with only a few of the sugars. When the rotation of one form is known, the rotation of the other may be calculated by solubility relations according to a method developed by Hudson [57] (1904) and by Lowry [58] (1904). When α-d-glucose dissolves in water containing alcohol the solubility of this initial form, quickly attained, is measured. There then results a slow increase in solubility, appearing at a rate equal to that of the speed of mutarotation. The equilibrium solubility thus measures the combined concentrations of α- and β-forms, and the rotation of the β-form may be calculated. It is necessary, of course, to maintain an excess of the α-form in the solid phase in order to keep its solubility constant. This method depends upon the presence in the equilibrium mixture of only two forms in appreciable amount. When the method was applied to sugars which were accessible both in the α- and in the β-forms, the expected results were obtained. The β-isomer of d-mannose was the first known form of this sugar, and when Levene [59] (1923) succeeded in preparing the α-isomer, its rotation was in agreement with that calculated by Hudson and Yanovsky [60] (1917).

The kinetics of sugar mutarotation have been studied extensively by many workers, among whom Hudson, Lowry, Osaka, Riiber, and Isbell have been outstanding. The course of the optical rotation change

[55] Lowry, J. Chem. Soc., 75, 211 (1899).
[56] Tanret, Bull. soc. chim., [3] 13, 728 (1895).
[57] Hudson, J. Am. Chem. Soc., 26, 1065 (1904).
[58] Lowry, J. Chem. Soc., 85, 1551 (1904).
[59] Levene, J. Biol. Chem., 57, 329 (1923); 59, 129 (1924).
[60] Hudson and Yanovsky, J. Am. Chem. Soc., 39, 1013 (1917).

in general follows the unimolecular law. An equation for calculating the velocity constant has been developed which does not require a knowledge of the molecular rotation of the second form.

$$\alpha\text{-form} \overset{k_1}{\underset{k_2}{\rightleftarrows}} \beta\text{-form}$$
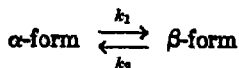
$K = k_1 + k_2 = \dfrac{1}{t} \log \dfrac{r_0 - r_\infty}{r_t - r_\infty}$, wherein K is the resultant velocity constant; k_1 and k_2 are the velocity constants of the two opposing reactions; t = time; r_0 = initial rotation; r_∞ = final rotation; r_t = rotation at time t.

For d-glucose, Hudson and Dale [61] (1917) found the value $k_1 + k_2$ = 0.00625 in water at 20° (minutes and decimal logarithms). This constant is identical for both forms of the sugar. The velocity of mutarotation is greatly accelerated by acids and bases, and this point was studied by Osaka [62] (1900) and by Hudson. The relation for this effect was given by Hudson [63] (1907) for d-glucose in water in the form:

$$K_{25°} = 0.0096 + 0.258 \,[\text{H}^+] + 9750 \,[\text{OH}^-]$$

This equation indicates that the acceleration of mutarotation by hydrogen and hydroxyl ions is directly proportional to their concentration and that the catalytic activity of hydroxyl ions is about 40,000 times as great as that of hydrogen ions. From the constant terms in the above equation and the velocity of mutarotation of glucose in pure water, Hudson [64] (1909) calculated the dissociation constant of water as 1.0 × 10^{-14}, which is in good agreement with the values obtained by other methods. It is of interest to note here that further studies on the influence of acids and bases on the velocity of mutarotation of glucose played a considerable part in the establishment by Lowry [65] (1923) and by Brönsted [66] (1923) of our present general theory of the nature of acids and bases.

Lowry has shown that mutarotation is not effected without a catalyst and that both a proton donor and acceptor are required. He has also shown that the phenomenon of mutarotation is not confined to the sugar group and that only an amphoteric solvent, such as water, is a true catalyst for mutarotation. Lowry and Faulkner [67] (1925) showed that

[61] Hudson and Dale, *ibid.*, **39**, 320 (1917).
[62] Osaka, *Z. physik. Chem.*, **35**, 661 (1900).
[63] Hudson, *J. Am. Chem. Soc.*, **29**, 1572 (1907).
[64] Hudson, *ibid.*, **31**, 1136 (1909).
[65] Lowry, *Chemistry & Industry*, **42**, 43 (1923).
[66] Brönsted, *Rec. trav. chim.*, **42**, 718 (1923).
[67] Lowry and Faulkner, *J. Chem. Soc.*, **127**, 2883 (1925).

the mutarotation of tetramethylglucose (p. 1554) could be arrested in a pyridine (weak base) solution and in a cresol (weak acid) solution, but that a mixture of these two solvents gave a velocity twenty times as great as that of water. Lowry terms mutarotation a prototropic change and attributes it to a proton shift in which the solvent plays a part. He interprets the change of an α- to a β-sugar as passing through an intermediate acyclic form (in water, the aldehydrol).

$$\text{H}\diagdown\diagup\text{OH} \quad \overset{\text{HOH}}{\underset{}{\rightleftarrows}} \quad \text{HC}\diagup^{\text{OH}} \quad \overset{\text{HOH}}{\underset{}{\rightleftarrows}} \quad \text{HO}\diagdown\diagup\text{H}$$
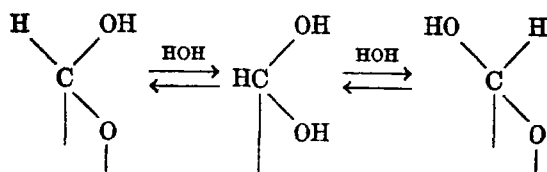
The deviation from the monomolecular law of the first part of the galactose mutarotation curve lends itself to this interpretation, and Smith and Lowry [68] (1928) have treated the data from the standpoint of a three-membered equilibrium. Evidence that more than two forms of a sugar are involved quite generally in mutarotation phenomena has also been obtained by Riiber [69] (1922 on) from studies of changes in volume and refractivity of sugar solutions undergoing mutarotation. Similar conclusions have been drawn by Isbell and Pigman [70] (1933 on) from precise optical rotatory measurements.

The known crystalline ketoses (Table VI, p. 1588) exhibit abnormal mutarotation phenomena. d-Fructose displays an enormous temperature effect, sorbose shows a bare trace of mutarotation,[71] and tagatose shows none. The aldopentose ribose has a rapid and anomalous mutarotation.[72] Thus it would appear that the tautomeric phenomena traced by these changes in optical rotatory power are not of the same nature throughout the sugar series.

α-, β-Isomerism. The preceding discussion of the phenomenon of mutarotation certainly establishes the fact that d-glucose, in common with all the mutarotating sugars, exists in at least two isomeric forms, the ordinary or α-d-glucose and the second or β-d-glucose. E. F. Armstrong [73] (1903) was able to relate these two forms of glucose to the two methylglucosides of Fischer by means of a very simple and beautiful

[68] Smith and Lowry, ibid., 666 (1928).
[69] Riiber, Tids. Kjemi Bergvesen, 12, 227 (1932) [C.A., 27, 953 (1933)], summarizing paper.
[70] Cf. Isbell and Pigman, J. Research Natl. Bur. Standards, 20, 773 (1938).
[71] Pigman and Isbell, ibid., 19, 443 (1937).
[72] Phelps, Isbell, and Pigman, J. Am. Chem. Soc., 56, 747 (1934).
[73] Armstrong, J. Chem. Soc., 83, 1305 (1903).

experiment. Fischer [74] (1894) had found that α-methylglucoside was hydrolyzed by the enzyme maltase and the β-isomer by emulsin. Armstrong simply observed these enzymatic hydrolyses polarimetrically and established the fact that the α-glucoside liberated initially the higher rotatory form of glucose and the β- the lower or β-glucose. Behrend and Roth [75] (1904) also related α- and β-glucose to the two known glucose pentaacetates by acetylation with pyridine and acetic anhydride at 0° α-Glucose produced α-glucose pentaacetate ($[\alpha]_D + 102°$, $CHCl_3$) and β-glucose yielded the β-pentaacetate ($[\alpha]_D + 4°$, $CHCl_3$).

Thus the presence of a ring structure in d-glucose was established There remained the problem of establishing upon good experimental evidence two points: first, the point of ring closure; and second, the configuration of carbon one.

Hudson [76] (1909) has given an empirical rule for designating α-, β isomers. Of an α-, β-pair of sugars in the d-series, he terms the α- that one which has the higher dextro rotation and assigns the hydroxyl to the right.
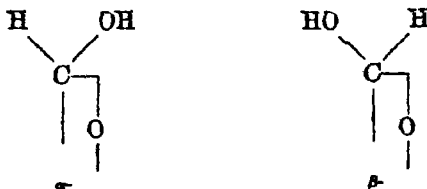
The reverse of course holds for the l-series, the enantiomorph of α-d glucose being designated α-l-glucose.

The knowledge of the α-, β-isomerism of sugars and their derivatives which is now available is due mainly to the work of Hudson and his co-workers. Hudson and Yanovsky [77] (1917) measured the rotation values of the unknown forms of the sugars by the maximum solubility method, but Hudson turned to the acetates of the sugars for a more convenient and richer source of pure α-, β-isomers. The dextro or α-glucose pentaacetate had been prepared by Franchimont [78] (1879; 1892), and the β- by Erwig and Koenigs [79] (1889). They have the following structures, in which the ring assignment was demonstrated later by methods yet to be described.

[74] Fischer, *Ber.*, **27**, 2985 (1894).

[75] Behrend and Roth, *Ann.*, **331**, 359 (1904); Hudson and Dale, *J. Am. Chem. Soc.*, **37**, 1264 (1915).

[76] Hudson, *J. Am. Chem. Soc.*, **31**, 66 (1909).

[77] Hudson and Yanovsky, *ibid.*, **39**, 1013 (1917).

[78] Franchimont, *Ber.*, **12**, 1940 (1879); *Rec. trav. chim.*, **11**, 106 (1892).

[79] Erwig and Koenigs, *Ber.*, **22**, 2207 (1889).

$$\begin{array}{c} H \diagdown \diagup OAc \\ C \\ | \\ HCOAc \\ | \\ AcOCH \\ | \\ HCOAc \\ | \\ HCO \\ | \\ CH_2OAc \end{array}$$

α-d-Glucose
pentaacetate

$$\begin{array}{c} AcO \diagdown \diagup H \\ C \\ | \\ HCOAc \\ | \\ AcOCH \\ | \\ HCOAc \\ | \\ HCO \\ | \\ CH_2OAc \end{array}$$

β-d-Glucose
pentaacetate
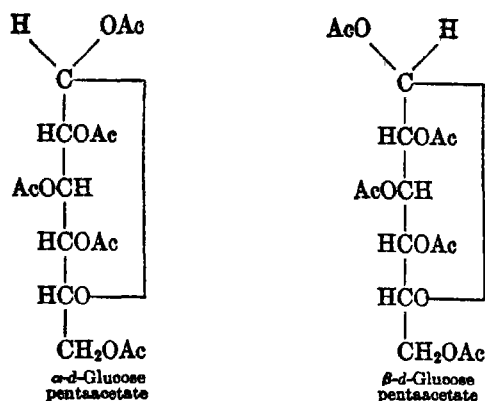
The methylglycosides (glycose referring to any sugar) and their acetates were also included in Hudson's studies.

It will be of interest to describe briefly the methods used in obtaining isomeric sugar acetates. When the two crystalline forms of a sugar are known, the previously cited Behrend acetylation at 0° with pyridine and acetic anhydride serves admirably.[75] The β-isomer (for the d-series) is obtained by acetylation of either form of the free sugar with hot acetic anhydride and sodium acetate. The β-isomer is transformed to the α-form by heating with acetic anhydride and zinc chloride, a reaction due to Erwig and Koenigs [79] (1889) but correctly interpreted and greatly extended by Hudson.

$$\alpha\text{-}d\text{-Glucose} \xrightarrow[\text{pyridine}]{Ac_2O} \alpha\text{-}d\text{-Glucose pentaacetate}$$

$$\xrightarrow[\text{NaOAc}]{Ac_2O}$$

$$\beta\text{-}d\text{-Glucose} \xrightarrow[\text{pyridine}]{Ac_2O} \beta\text{-}d\text{-Glucose pentaacetate}$$

(with $\xrightarrow[\text{ZnCl}_2]{Ac_2O}$ connecting β-pentaacetate to α-pentaacetate)
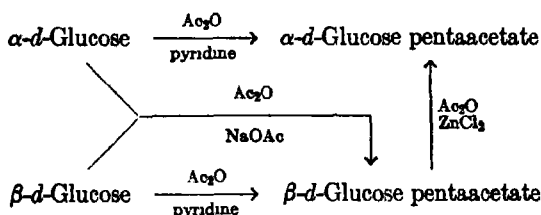
Rules of Optical Rotation. Hudson became greatly interested in a study of the numerical values of optical rotations, being especially concerned with testing the van't Hoff theory of optical superposition in the sugar group. Hudson [80] (1909) developed two rules which he termed the rules of isorotation. If the rotation contributed by carbon one is termed A and that of the remaining asymmetric centers B, then the molecular rotations will be:

for the α-form (d-series),   A + B
"   " β-form   "   , −A + B

[80] Hudson, *J. Am. Chem. Soc.*, **31**, 66 (1909).

It follows from the above that in an α-, β- pair of isomers, the sum (2B) of their molecular rotations will be a constant (rule 1) characteristic of the particular sugar, and the difference (2A) will be a constant (rule 2) characteristic of the nature of the hydroxyl group or substituted hydroxyl on carbon one. These modified rules of optical superposition apparently do not have a rigid general application but hold remarkably well with many closely related structures, as is illustrated in Tables I, II, and V (pp. 1553 and 1582).

Hudson's rules of isorotation having been briefly discussed, a number of other rules based upon optical properties may be cited. Hudson [81] (1910) observed that, in the ordinary γ-lactones of the aldonic acids, the sign of rotation of the lactone was determined by the spatial configuration of the asymmetric center (carbon four) where lactonization took place. If carbon four was $(+)$, hydroxyl on the right, the lactone was dextrorotatory; and if carbon four was $(-)$, hydroxyl on the left, the lactone showed a levorotation. Levene [82] (1915) obtained evidence to show that, when carbon two in a sugar acid is $(+)$, the ion (rotation of the salt) is more dextrorotatory than the slightly dissociated free acid, and vice versa. It was also noted that the sign of rotation of the phenyl-hydrazides of the sugar acids was determined by the sign of carbon two: when this was $(+)$ the hydrazide was dextrorotatory, and vice versa.

TABLE I

OPTICAL ROTATIONS OF ACETYLATED SUGARS IN CHLOROFORM

Substance	Molecular Rotation of α-form	Molecular Rotation of β-form	2A (Difference)	2B (Sum)
l-Arabinose tetraacetate *	$+13,500°$	$+46,800°$	$-33,300$	$+60,300$
d-Xylose tetraacetate	$+28,300$	$-7,900$	$+36,200$	$+20,400$
d-Glucose pentaacetate	$+39,600$	$+1,500$	$+38,100$	$+41,100$
d-Mannose pentaacetate	$+21,500$	$-9,800$	$+31,300$	$+11,700$
d-Galactose pentaacetate	$+41,700$	$+9,000$	$+32,700$	$+50,700$
d-[α-Glucoheptose] hexaacetate	$+40,200$	$+2,200$	$+38,000$	$+42,400$
d-Glucosamine pentaacetate	$+36,400$	$+470$	$+35,930$	$+36,900$
d-Chondrosamine pentaacetate	$+39,400$	$+4,100$	$+35,300$	$+43,500$
Cellobiose octaacetate	$+27,800$	$-9,900$	$+37,700$	$+17,900$
Gentiobiose octaacetate	$+35,500$	$-3,600$	$+39,100$	$+31,900$
Lactose octaacetate	$+36,500$	$-2,900$	$+39,400$	$+33,600$
Maltose octaacetate	$+83,000$	$+42,500$	$+40,500$	$+125,500$

* The negative sign of 2A is due to this being an l-sugar.

[81] Hudson, *ibid.*, **32**, 338 (1910); *cf.* Hudson, *ibid.*, **61**, 1525 (1939).

[82] Levene, *J. Biol. Chem.*, **23**, 145 (1915).

TABLE II

OPTICAL ROTATIONS OF ACETYLATED METHYLGLYCOSIDES IN CHLOROFORM

Substance	Molecular Rotation of α-form	Molecular Rotation of β-form	2A (Differ- ence)
Methyl-d-xyloside triacetate...	+34,700°	−17,600°	+52,300
Methyl-d-glucoside tetraacetate.. ..	+47,300	−6,600	+53,900
Methyl-d-galactoside tetraacetate	+48,400	−5,100	+53,500
Methylgentiobioside heptaacetate.	+41,900	−12,350	+54,250
Methylcellobioside heptaacetate.......	+36,200	−16,500	+52,700
Methyl-d-guloside tetraacetate..	+35,200	−11,600	+46,800
Methyl-d-[α-glucoheptoside] pentaacetate.. .	+39,500	−6,900	+46,400
Methyl-d-mannoside tetraacetate... . .	+17,800	−18,100	+35,900
Methyl-l-rhamnoside triacetate *	−16,300	+13,900	−30,200

* Measured in acetylene tetrachloride solution.

This is known as the hydrazide rule of Levene and Hudson. Hudson [83] (1918) found that it applies likewise to the sugar amides, and Deulofeu [84] (1933) has shown that it also applies to the acetylated nitriles of the sugar acids. These rules are of a more qualitative nature than the isorotation rules and have found wide application. In particular, the lactone rule of Hudson has been of great value. For example, Clark [85] (1922) determined the configuration of carbon five in the methylpentose l-fucose by a clever application of the lactone rule. Anderson [86] (1912) has shown that β-d-metasaccharonolactone (p. 1646) rotates slightly to the left (−4.7°) and is in disagreement, therefore, with the relation between rotation and structure because its γ-ring is to the right of the structure. As Anderson points out, however, β-saccharonic acid is strongly levorotatory, and the change of rotation due to lactone formation is in the direction called for by theory. The same explanation probably holds for the small levorotation of d-allonolactone (−6°), which is in the opposite direction to that indicated by theory.

Establishment of the Pyranose Ring Structure. In 1915–1916 Hudson [87] and his co-workers Parker and Johnson were investigating the α- and β-pentaacetates of d-galactose and obtained four crystalline isomers,

[83] Hudson, J. Am. Chem. Soc., 40, 813 (1918).
[84] Deulofeu, Nature, 131, 548 (1933).
[85] Clark, J. Biol. Chem., 54, 65 (1922).
[86] Anderson, J. Am. Chem. Soc., 34, 51 (1912).
[87] Hudson and Parker, ibid., 37, 1589 (1915); Hudson and J. M. Johnson, ibid., 38, 1223 (1916).

corresponding with two α-, β-pairs. This represented excellent evidence, based upon crystalline derivatives, that more than one ring form could exist in a sugar and could be explained on the basis of ring closure on different carbon atoms. Similar compounds were not obtained in the glucose series until 1927 when Schlubach and Huntenburg [88] added the third and fourth pentabenzoates of d-glucose to the two previously prepared by Skraup [89] (1889) and by Fischer and co-workers [90] (1911; 1912). Sugar benzoates are rather difficult to purify, and Levene and Meyer [91] (1928) were able to change considerably the constants given by Fischer and by Schlubach for these compounds.

The determination of the size of the oxide ring in sugar derivatives was first accomplished by means of methylation studies. Purdie had developed a workable method for obtaining methyl ethers of hydroxy acids, which consisted in reacting the alcoholic substance with methyl iodide and silver oxide. In 1903 Purdie and Irvine [92] published the results of their extension of this reaction to α-methyl-d-glucoside. A pentamethyl derivative was obtained which could be distilled in a good vacuum and which on hydrolysis lost the glycosidic (carbon one) methyl group and produced a crystalline tetramethylglucose. The latter fact was indeed fortunate, as this substance still remains one of the few methylated sugars which crystallizes with any ease. In this derivative the hydroxyls are blocked with stable ether groups, and many results could now be obtained which were not possible with substituents less resistant to chemical action.

An alternative methylation procedure applicable to the sugar series is that employing methyl sulfate and alkali. This general method of etherification was first recorded by Ulmann and Wenner [93] (1900); it was applied to the methylation of cellulose by Denham and Woodhouse [94] (1913); and was shown to be suitable for the methylation of glycosides by Haworth [95] (1915).

Tetramethylglucose is a substance which can be oxidized, and from a study of its oxidation products the point of ring closure may be ascertained. Hirst [96] (1926) oxidized tetramethylglucose with nitric acid and

[88] Schlubach and Huntenburg, *Ber.*, **60**, 1487 (1927).

[89] Skraup, *Monatsh.*, **10**, 395 (1889).

[90] Fischer and Helferich, *Ann.*, **383**, 68 (1911); Fischer and K. Freudenberg, *Ber.*, **45** 2724 (1912).

[91] Levene and Meyer, *J. Biol. Chem.*, **76**, 513 (1928).

[92] Purdie and Irvine, *J. Chem. Soc.*, **83**, 1021 (1903).

[93] Ulmann and Wenner, *Ber.*, **33**, 2476 (1900).

[94] Denham and Woodhouse, *J. Chem. Soc.*, **103**, 1735 (1913).

[95] Haworth, *ibid.*, **107**, 13 (1915).

[96] Hirst, *ibid.*, 350 (1926).

$$
\begin{array}{c}
\overset{H\;\;\;\;OH}{\underset{\diagdown\;\diagup}{C}} \\
| \\
HCOH \\
| \\
HOCH \\
| \\
HCOH \\
| \\
HCO \\
| \\
CH_2OH
\end{array}
\quad\longrightarrow\quad
\begin{array}{c}
\overset{H\;\;\;\;OMe}{\underset{\diagdown\;\diagup}{C}} \\
| \\
HCOH \\
| \\
HOCH \\
| \\
HCOH \\
| \\
HCO \\
| \\
CH_2OH
\end{array}
\quad\xrightarrow[\text{4MeI}]{\text{2Ag}_2\text{O}}\quad
\begin{array}{c}
\overset{H\;\;\;\;OMe}{\underset{\diagdown\;\diagup}{C}} \\
| \\
HCOMe \\
| \\
MeOCH \\
| \\
HCOMe \\
| \\
HCO \\
| \\
CH_2OMe
\end{array}
\quad + 4AgI + 2H_2O
$$

α-d-Glucose   α-Methyl-d-glucoside   Tetramethyl-α-methyl-d-glucoside

$$\downarrow \begin{array}{c}\text{HOH}\\(\text{HCl})\end{array}$$

$$
\begin{array}{c}
\overset{H\;\;\;\;OH}{\underset{\diagdown\;\diagup}{C}} \\
| \\
HCOMe \\
| \\
MeOCH \\
| \\
HCOMe \\
| \\
HCO \\
| \\
CH_2OMe
\end{array}
\quad + MeOH
$$

Tetramethyl-d-glucose
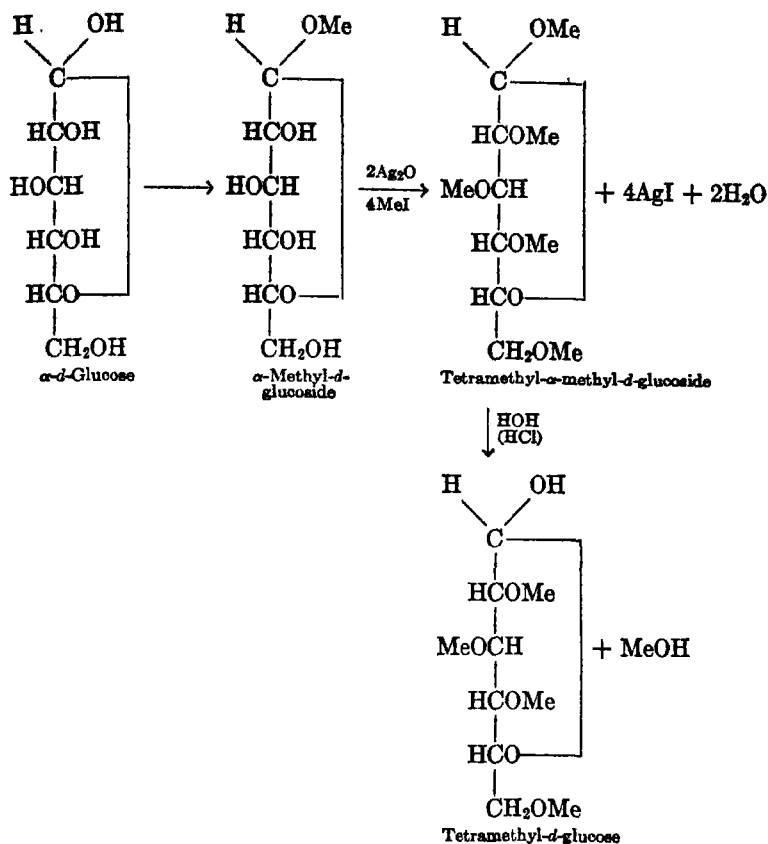
identified the acids formed by means of their crystalline diamides. He identified *i*-trimethoxyxyloglutaric acid and (dextro)-dimethoxysuccinic acid among the oxidation products and thus established the fact that the ring closure in tetramethylglucose was on carbon five. The diamide of this methylated tartaric acid had been characterized previously by Purdie and Irvine [97] (1901).

Since Armstrong had related α-d-glucose to α-methylglucoside, α-glucose itself has the same ring structure as tetramethylglucose, provided that no ring shift occurred during the methylation process. Since β-methylglucoside likewise yields the same tetramethylglucose, α- and β-glucose have the same ring structure. This is variously denoted as (1,5-), amylene oxidic, normal or pyranose. The last name was suggested by Haworth and is preferable. Accordingly, the ordinary crystalline form

[97] Purdie and Irvine, *ibid.*, 79, 960 (1901).

$$
\begin{array}{c}
\text{H} \quad \text{OH} \\
\diagdown \diagup \\
\text{C} \\
| \\
\text{HCOMe} \\
| \\
\text{MeOCH} \\
| \\
\text{HCOMe} \\
| \\
\text{HCO} \!-\!\!\! \\
| \\
\text{CH}_2\text{OMe} \\
\text{Tetramethylglucose}
\end{array}
\rightarrow
\left[
\begin{array}{c}
\text{CO}_2\text{H} \\
| \\
\text{HCOMe} \\
| \\
\text{MeOCH} \\
| \\
\text{HCOMe} \\
| \\
\text{C}\!=\!\text{O} \\
| \\
\text{CH}_2\text{OMe}
\end{array}
\right]
$$

Upper branch:

$$
\nearrow
\left[
\begin{array}{c}
\text{CO}_2\text{H} \\
| \\
\text{HCOMe} \\
| \\
\text{MeOCH} \\
| \\
\text{HCOMe} \\
| \\
\text{COH} \\
\| \\
\text{CHOMe}
\end{array}
\right]
\rightarrow
\begin{array}{c}
\text{CO}_2\text{H} \\
| \\
\text{HCOMe} \\
| \\
\text{MeOCH} \\
| \\
\text{HCOMe} \\
| \\
\text{CO}_2\text{H} \\
\textit{i}\text{-Trimethoxy-} \\
\text{xyloglutaric acid}
\end{array}
$$

Lower branch:

$$
\searrow
\left[
\begin{array}{c}
\text{CO}_2\text{H} \\
| \\
\text{HCOMe} \\
| \\
\text{MeOCH} \\
| \\
\text{COMe} \\
\| \\
\text{COH} \\
| \\
\text{CH}_2\text{OMe}
\end{array}
\right]
\rightarrow
\begin{array}{c}
\text{CO}_2\text{H} \\
| \\
\text{HCOMe} \\
| \\
\text{MeOCH} \\
| \\
\text{CO}_2\text{H} \\
\text{(dextro)-Dimeth-} \\
\text{oxysuccinic acid}
\end{array}
$$

of glucose is accurately named α-*d*-glucopyranose and has the following structure:

$$
\text{CH}_2\text{OH}\!-\!\!
\overset{\text{H}}{\underset{\text{O}}{\text{C}}}\!-\!\!
\overset{\text{H}}{\underset{\text{OH}}{\text{C}}}\!-\!\!
\overset{\text{OH}}{\underset{\text{H}}{\text{C}}}\!-\!\!
\overset{\text{H}}{\underset{\text{OH}}{\text{C}}}\!-\!\!
\overset{\text{H}}{\underset{\text{OH}}{\text{C}}}\!\!-\!\!
$$
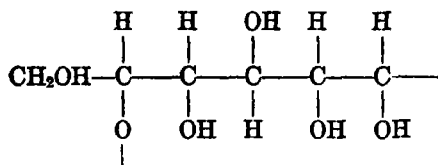
Haworth correctly considers that the true spatial relationships are better shown by a hexagonal formula, and this type of representation has been widely adopted, especially for depicting the disaccharide and polysaccharide molecules. X-ray evidence in support of such a ring has been obtained.[98]
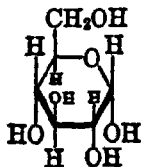
Establishment of the Furanose Ring Structure. The pyranose ring structure established for α- and β-*d*-glucose has been extended to other

sugars, and it has been determined that the normal or ordinary forms of the sugars and their derivatives possess the pyranose ring. Other ring structures are possible, however. In 1932 Haworth [99] reported the synthesis of a third crystalline methylglucoside which contained a (1,4-) or furanose ring. Substances containing this unstable ring have also been termed butylene oxidic or γ-sugars. In place of the name ring, the term lactol has been suggested by Helferich, this name being analogous to lactone. As the above glucofuranoside was synthesized from monoacetoneglucose, it is necessary to digress sufficiently to discuss the structure of this ketone condensation product of glucose. In working with the sugar alcohols, Meunier [100] (1888) had characterized them as their benzal derivatives, in which the benzaldehyde had undergone acetal formation with the polyhydroxy sugar alcohol to form a cyclic acetal. This reaction is general for polyhydroxy compounds and is effected by treatment of the substance with the aldehyde or ketone in the presence of an acidic dehydrating agent such as zinc chloride or sulfuric acid. In 1895 Fischer [101] obtained a crystalline derivative of glucose in which two moles of acetone had reacted with the glucose. This substance is known as diacetoneglucose, and, on graded acid hydrolysis, crystalline monoacetoneglucose is formed. The structure of these two compounds has been the subject of a number of investigations.

Irvine and Scott [102] (1913) methylated diacetoneglucose, and after removal of the acetone groups they obtained a beautifully crystalline monomethylglucose which formed a crystalline monomethyl phenylosazone. They also obtained a syrupy trimethylglucose from monoacetoneglucose. Neither of the two unsubstituted acetoneglucoses reduces Fehling's solution. These facts place the acetone group of monoacetoneglucose on positions one and two. Levene and Meyer [103] (1922) oxidized this monomethylglucose with nitric acid and obtained a crystalline monomethylglucosaccharolactone, thus eliminating positions one and six for the methyl substituent.

The allocation of position three for the open hydroxyl of diacetoneglucose was made by K. Freudenberg and by Levene through the use of entirely different methods of proof. K. Freudenberg and Doser [104] (1923) converted diacetoneglucose to the previously known 3-pyrazolecarboxylic acid through the following steps, all products being crystalline.

[99] Haworth, Porter, and Waine, J. Chem. Soc., 2254 (1932).
[100] Meunier, Compt. rend., 106, 1425 (1888).
[101] Fischer, Ber., 28, 1165 (1895).
[102] Irvine and Scott, J. Chem. Soc., 103, 570 (1913).
[103] Levene and Meyer, J. Biol. Chem., 54, 805 (1922).
[104] Freudenberg and Doser, Ber., 56, 1243 (1923).

$$
\begin{array}{c}
\underset{\text{Diacetoneglucose}}{
\left[
\begin{array}{l}
\text{HCO} \\
\text{HCO} \\
\text{HOCH}
\end{array}
\right\rangle\!\text{CMe}_2
}
\quad \rightarrow \quad
\underset{\substack{\text{Diacetoneglucose} \\ \text{p-toluenesulfonate}}}{
\text{CH}_3\!\!\left\langle\!\!\bigcirc\!\!\right\rangle\!\!\text{SO}_2\text{OCH}\Big|
\left[
\begin{array}{l}
\text{HCO} \\
\text{HCO}
\end{array}
\right\rangle\!\text{CMe}_2
}
\xrightarrow{\text{NH}_2\text{NH}_2}
\underset{\substack{\text{Diacetonehydrazino-} \\ \text{glucose}}}{
\text{H}_2\text{NHNCH}\Big|
\left[
\begin{array}{l}
\text{HCO} \\
\text{HCO}
\end{array}
\right\rangle\!\text{CMe}_2
}
\end{array}
$$

$$
\xrightarrow{\text{HCl}} \text{HCl}\cdot
\underset{\text{Glycerylpyrazole}}{
\begin{array}{l}
\text{N}\!\!=\!\!\text{CH} \\
\quad\ \ |\quad |\ \ \text{CH} \\
\text{HN}\!\!-\!\!\text{C} \\
\quad\ \ \text{HCOH} \\
\quad\ \ \text{HCOH} \\
\quad\ \ \text{CH}_2\text{OH}
\end{array}
}
\xrightarrow{\text{KMnO}_4}
\underset{\text{3-Pyrazolecarboxylic acid}}{
\text{CH}\!\!\left\langle\!\!\begin{array}{l}\text{NH}\!\!-\!\!\text{N} \\ \\ \text{CH}\!\!-\!\!\text{C}\!\!-\!\!\text{CO}_2\text{H}\end{array}\right.
}
$$
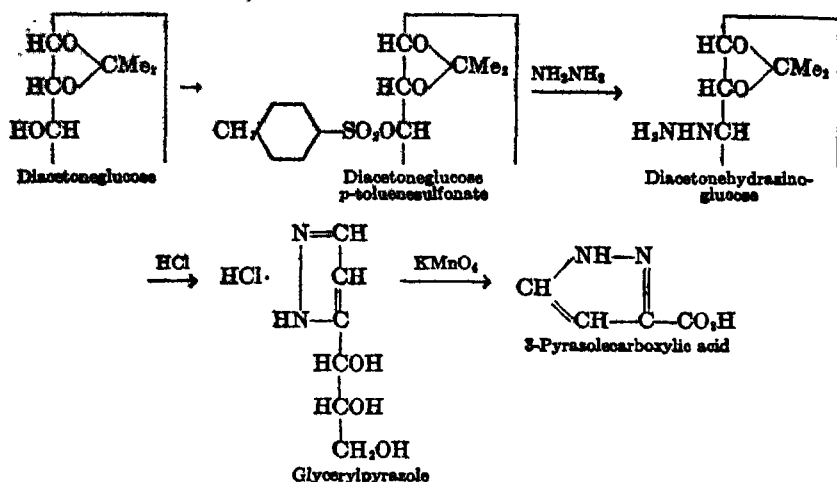
Levene and Meyer [105] (1924) converted the monomethylglucose of Irvine and Scott to a crystalline monomethylglucoheptonolactone through the cyanohydrin reaction. This lactone was dextrorotatory (+48°) whereas the lactone of d-α-glucoheptonic acid is levorotatory (−56°). Therefore, in accordance with Hudson's lactone rule, the single (−) carbon (hydroxyl on left) of glucose is occupied by a methoxyl group in the monomethylglucose, and this (−) carbon atom is known to be number three. Levene and Simms [106] (1925) showed that the above 4-methylglucoheptonolactone was an unstable or δ-lactone.

The above work gives the structure of the first three carbons of diacetoneglucose, but does not establish the nature of the remainder. Levene and Meyer [107] (1926), and also Micheel and Hess [108] (1926), further methylated the syrupy trimethylglucose prepared from mono-acetoneglucose and obtained a ring isomer of tetramethylglucopyranose. Micheel and Hess reported their final product as crystalline and melting a little above 0°. The furanose nature of this ring was proved definitely by Anderson, Charlton, and Haworth [109] (1929) by oxidation to 2,3,5,6-tetramethylgluconic acid, isolated as its crystalline γ-lactone and crystalline phenylhydrazide. The fact that the acetone group of mono-acetoneglucose is placed on carbon atoms one and two was established beyond doubt by the isolation of a crystalline trimethylglucose phenyl-osazone of the trimethylglucose by these workers. Accordingly, it is now

[105] Levene and Meyer, J. Biol. Chem., 60, 173 (1924).
[106] Levene and Simms, ibid., 65, 31 (1925).
[107] Levene and Meyer, ibid., 70, 343 (1926).
[108] Micheel and Hess, Ann., 450, 21 (1926).
[109] Anderson, Charlton, and Haworth, J. Chem. Soc., 1329 (1929).

apparent that, in diacetoneglucose, positions one and two carry an acetone group; three is open, and the lactol ring is on carbon four. This leaves positions five and six for the second acetone group. It is of interest that a furanose (1,4-) derivative is thus directly obtained from an acid solution of glucose.

Diacetoneglucose   Monoacetoneglucose
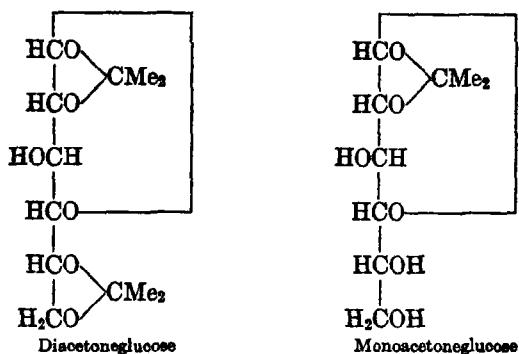
The above proof of structure does not involve the unwarranted assumption that acetone reacts only with hydroxyl groups that are adjacent. This assumption has been definitely disproved by the thorough studies of Hibbert and co-workers on glycerol cyclic acetals. He has shown that a partition is established between the five- and six-membered cyclic acetals. This variation in ring size has a bearing on sugar lactol structure, as sugar lactols are really five- and six-membered cyclic hemiacetals.

Monoacetoneglucose is a non-reducing structure in which the furanose ring is stabilized. By reacting this substance (I) with phosgene, Haworth and Porter [110] (1929) obtained a crystalline 5,6-carbonate of monoacetoneglucose (II). The structure of this substance was proved by converting its p-toluenesulfonate into the known p-toluenesulfonate of diacetoneglucose. In this mixed carbonate and cyclic acetal of glucose, Haworth possessed a compound wherein the acetone group was sensitive to acidity and stable to alkali, whereas the carbonate ester group had the reverse reactivity. Reaction of this substance (II) with methanol and hydrogen chloride resulted in the loss of the acetone group and formation of the crystalline α- and β-methylglucofuranoside-5,6-carbonates (III), the furanose ring structure being meanwhile stabilized by the carbonate group. These were separated, and mild saponification produced the glucofuranosides (IV), of which only the α-form was obtained crystalline. This work was completed by Haworth, Porter, and

[110] Haworth and Porter, *ibid.*, 2796 (1929).

Waine [111] in 1932. In the case of the similar ethylglucofuranosides, Haworth and Porter [110] (1929) succeeded in obtaining both α- and β-forms in crystalline condition, the separation being effected through fractionation of their crystalline 5,6-carbonate-2,3-diacetates.
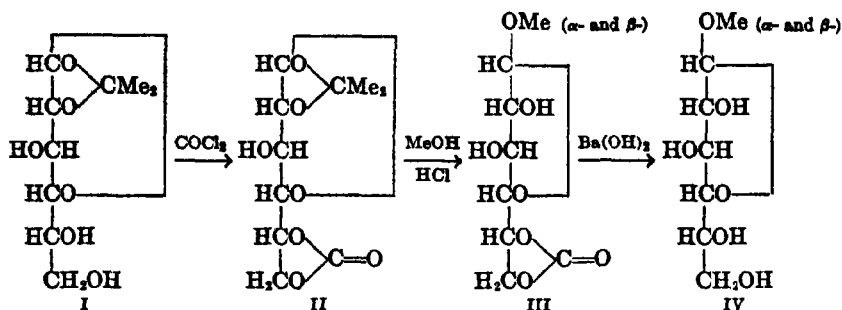
These furanosides are characterized by their ease of hydrolysis with acids, and the ring is accordingly very labile. They are not affected by dilute permanganate or by Fehling's solution. Previous statements that such behavior was characteristic of γ-glycosides were thus shown to be in error, easily oxidizable impurities being present in the older syrupy preparations.

Complete methylation of α-methylglucofuranoside followed by hydrolysis of the glycosidic methyl group produces the liquid tetramethylglucofuranose or γ-tetramethylglucose. This substance on oxidation with hypobromite forms a crystalline lactone, which in turn may be converted to a crystalline phenylhydrazide. Nitric acid oxidation of this lactone by Haworth, Hirst, and Miller [112] (1927) yielded (dextro)-dimethoxysuccinic acid, isolated as the crystalline amide and methylamide.
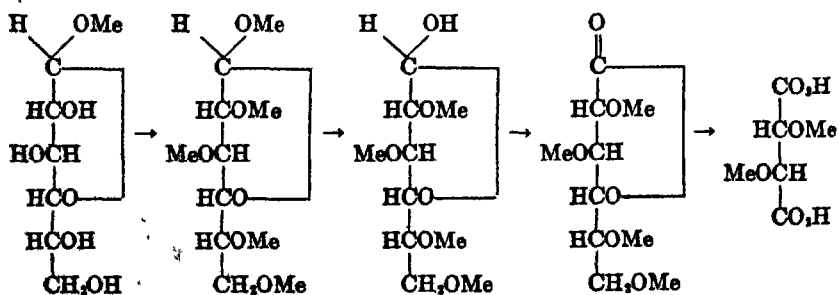
The α- and β-glucofuranose pentabenzoates of Schlubach are well-

[111] Haworth, Porter, and Waine, *ibid.*, 2254 (1932).
[112] Haworth, Hirst, and Miller, *ibid.*, 2436 (1927).

characterized crystalline derivatives of glucofuranose. Their structure follows from their method of preparation from monoacetoneglucose, in which the furanose ring is established by the experiments previously cited. Fischer and Rund [113] (1916) had benzoylated monoacetoneglucose and selectively hydrolyzed the acetone group with hydrochloric acid, thus producing the tribenzoate, which was isolated as a crystalline carbon tetrachloride addition compound. Fischer was merely interested in obtaining a partially benzoylated sugar and of course was unaware that he had in hand a glucose derivative containing an unusual ring. The contribution of Schlubach [114] (1927) was to benzoylate this substance further and to separate and isolate the two isomeric pentabenzoates thus formed.

The methods used for obtaining benzoylated sugars may be mentioned briefly. By means of the Schotten-Baumann method using dilute alkali and benzoyl chloride, Kueny [115] (1890) and other workers obtained some of the first sugar esters. The difficulty with this method when applied to the sugars was that mixtures of partially benzoylated structures generally were formed. To obtain complete benzoylation of a sugar, Fischer [116] (1912) used successfully benzoyl chloride and quinoline. This procedure was later improved by substituting pyridine for the quinoline.
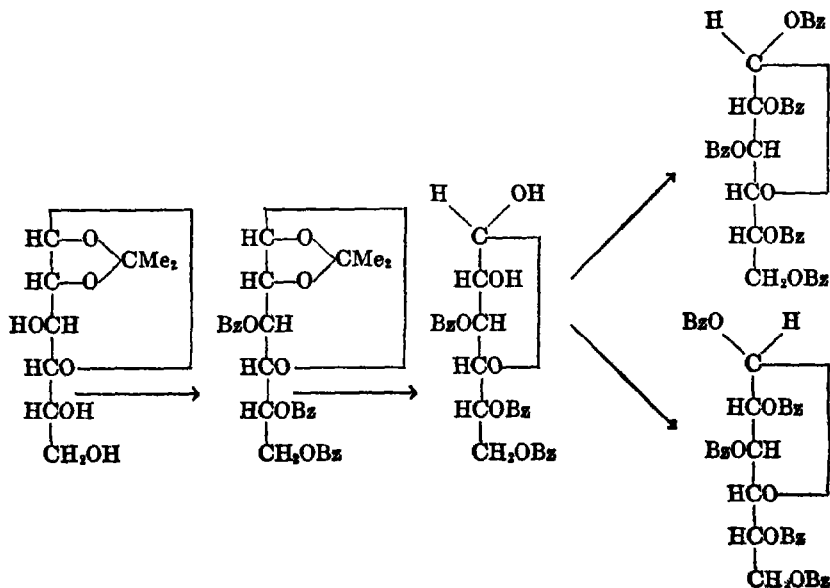
[113] Fischer and Rund, *Ber.*, **49**, 100 (1916).
[114] Schlubach and Huntenburg, *Ber.*, **60**, 1487 (1927).
[115] Kueny, *Z. physiol. Chem.*, **14**, 333 (1890).
[116] Fischer and K. Freudenberg, *Ber.*, **45**, 2724 (1912).

Tetramethylglucofuranose had been prepared in 1915 by Irvine [117] and his students through methylation and subsequent hydrolysis of the so-called γ-methylglucoside obtained by Fischer [118] in 1914 and recognized by him as a ring isomer of the ordinary or normal methylglucosides. The γ- (termed by the German workers h- for *hetero*) methylglucoside is the impure syrupy mixture which arises when glucose is allowed to react with methanol at room temperature in the presence of a considerable concentration of hydrogen chloride. This reaction is a general one for the reducing monosaccharides, and all the γ-glycosides so obtained are characterized by their ease of hydrolysis. Some of the normal glycosides are likewise formed in the reaction, and Levene, Raymond, and Dillon [119] (1932) have obtained data to show that the γ-glycosides are produced initially and then rearrange in part, under the experimental conditions, to form the more stable pyranosides. It is probable that this is not a true rearrangement but a hydrolysis followed by glycopyranoside formation. Very few crystalline isomers have been isolated from these γ-methylglycoside syrups. Hudson and co-workers have isolated a crystalline isomer from the reaction product with fructose [120] (1934) and with arabinose [121] (1937). Haworth [122] (1930) obtained crystalline α-methylmannofuranoside from mannose after he had obtained nuclei by an extension to mannose [123] of his carbonate work, so successfully used in obtaining the pure methylglucofuranoside. A fortunate point with mannose is that this sugar tends to form only one glycoside, the α-, and so the number of possible isomers present in the syrupy γ-methylmannoside was accordingly decreased. All the methylated furanose sugar preparations so far obtained through these γ-glycosides have been syrups. In the partially substituted sugar series, a crystalline 5-methyl-*l*-rhamnofuranose has been recorded. [124]

An important general procedure for the synthesis of furanosides has been established by Green and Pacsu [125] (1937). These workers found that, when a sugar mercaptal (p. 1575) is treated at the appropriate temperature with mercuric chloride and yellow mercuric oxide in the presence of an alcohol, a mixture of the α- and β-furanosides of the alcohol used may be obtained. The reaction sometimes leads to the formation of thiofuranosides (furanosides of thiols) or of acyclic acetals (p. 1578).

[117] Irvine, Fyfe, and Hogg, *J. Chem. Soc.*, **107**, 524 (1915).
[118] Fischer, *Ber.*, **47**, 1980 (1914).
[119] Levene, Raymond, and Dillon, *J. Biol. Chem.*, **95**, 699 (1932).
[120] Purves and Hudson, *J. Am. Chem. Soc.*, **56**, 708 (1934).
[121] Montgomery and Hudson, *ibid.*, **59**, 992 (1937).
[122] Haworth, Hirst, and Webb, *J. Chem. Soc.*, 651 (1930).
[123] Haworth and Porter, *ibid.*, 649 (1930).
[124] Levene and Compton, *J. Biol. Chem.*, **114**, 9 (1936).
[125] Green and Pacsu, *J. Am. Chem. Soc.*, **59**, 1205 (1937).

$$
\begin{array}{ccc}
\underset{\displaystyle\underset{\displaystyle\underset{\displaystyle CH_2OH}{\mid}}{(CHOH)_4}}{HC{<}^{SEt}_{SEt}}
& \xrightarrow[HgCl_2 + HgO]{MeOH}
& \underset{\displaystyle\underset{\displaystyle\underset{\displaystyle CH_2OH}{\mid}}{CHOH}}{\left.\begin{array}{c} OMe \;(\alpha\text{- and }\beta\text{-}) \\ HC \\ CHOH \\ CHOH \\ HCO \end{array}\right]} \quad + 2ClHgSEt + H_2O
\end{array}
$$
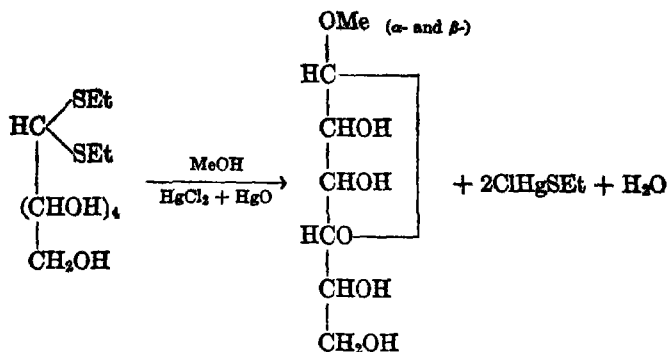
By means of the above method a number of crystalline furanosides have been made available. Thus, the first crystalline pair of methyl-furanosides in the sugar series was found for d-mannose [126] (1940), and the α- and β-forms of ethylgalactofuranoside were prepared.[127]

The tetrose sugars can exist in the monomolecular form only as a furanose or smaller ring. None of the tetrose sugars has so far been obtained crystalline. The molecular weight of an erythrose syrup has been found to be that of a normal monomer by Deulofeu [128] (1932). This might have been expected from the previous work of Helferich and his collaborators [129] (1921) with γ-hydroxy aldehydes. An outstanding advance in tetrose chemistry was the isolation of a crystalline diacetate of d-threose by W. Freudenberg [130] (1932) and of a crystalline triacetate of the same by Hockett [131] (1934). Swan and Evans [132] (1935) also obtained the first crystalline glycoside of a tetrose (α-methyl-l-arabomethyloside).

Lactone Studies Related to the Determination of Sugar Ring Structure. Any discussion of ring structure in the sugar series would be incomplete without a consideration of the supporting evidence obtained from the study of lactones. It has been mentioned how the early known γ-lactones of the sugar acids were important in synthetic work and also how a statistical study of their rotatory power led to the establishment of the lactone rule of Hudson. Some confusion entered this field when Nef and Hedenburg [133] in 1914 isolated a *second* crystalline lactone of gluconic acid and also a second of mannonic acid. It was obvious that

[126] Scattergood and Pacsu, *ibid.*, **62**, 903 (1940).

[127] Green and Pacsu, *ibid.*, **59**, 2569 (1937).

[128] Deulofeu, *J. Chem. Soc.*, 2973 (1932).

[129] Helferich and Lecher, *Ber.*, **54**, 930 (1921).

[130] W. Freudenberg, *Ber.*, **65**, 168 (1932).

[131] Hockett, *J. Am. Chem. Soc.*, **56**, 994 (1934).

[132] Swan and Evans, *ibid.*, **57**, 200 (1935).

[133] Nef, *Ann.*, **403**, 322 (1914); Hedenburg, *J. Am. Chem. Soc.*, **37**, 345 (1915).

one lactone in each of the two pairs was not a γ-lactone. Of the two lactones, one was much more unstable than the other, and this unstable form was apparently the one which was not the γ-lactone.

In 1925 Levene and Simms [124] published a very important paper in which they showed clearly that, when a free aldonic acid was liberated from an aqueous solution of its salt by the addition of one equivalent of mineral acid, lactonization took place in two stages. The first was a very rapid formation of an unstable lactone, followed by the slow formation of the stable γ-lactone and the disappearance of the unstable lactone. The final equilibrium mixture apparently contained the γ-lactone in equilibrium with the free acid. This reminds one of the results obtained later by Levene, Raymond, and Dillon in their studies of methylglycoside formation.

Levene and Simms [124] (1925) applied this procedure to the two methylated mannonic acids. The one acid (I) was obtained from the crystalline methylation product of the stable mannonic lactone and the other (II) by oxidation of normal tetramethylmannose. Acid II rapidly formed an unstable lactone in aqueous solution; acid I showed the slow formation of a stable, apparently (1,4-), lactone. The conclusion was reached then that normal tetramethylmannose and thus also the ordi-
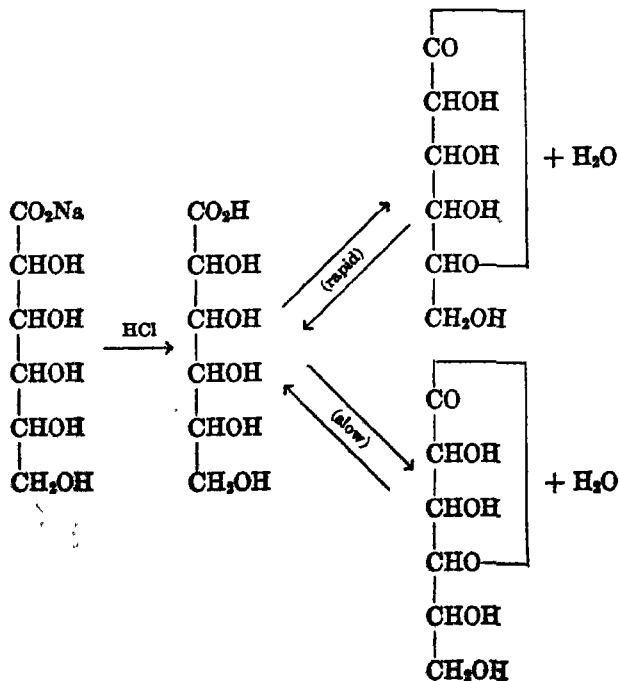
[124] Levene and Simms, J. Biol. Chem., 65, 31 (1925).

nary α-methylmannoside did not possess a (1,4-) ring but probably had a (1,5-) ring. In 1926, Levene and Simms [135] extended this work to the glucose series with similar results.

$$
\begin{array}{ccccc}
\text{CO}& \text{CO}& \text{CO}_2\text{Na}& \text{CO}_2\text{H}& \text{CO}\\
\text{HOCH}& \text{MeOCH}& \text{MeOCH}& \text{MeOCH}& \text{MeOCH}\\
\text{HOCH}& \rightarrow\ \text{MeOCH}& \rightarrow\ \text{MeOCH}& \rightarrow\ \text{MeOCH}& \rightleftarrows\ \text{MeOCH}\\
\text{HCO}& \text{HCO}& \text{HCOH}& \text{HCOH}& \text{HCO}\\
\text{HCOH}& \text{HCOMe}& \text{HCOMe}& \text{HCOMe}& \text{HCOMe}\\
\text{CH}_2\text{OH}& \text{CH}_2\text{OMe}& \text{CH}_2\text{OMe}& \text{CH}_2\text{OMe}& \text{CH}_2\text{OMe}\\
\gamma\text{-Mannonolactone}& & & & \text{I}
\end{array}
$$

$$
\begin{array}{cccc}
\underset{\text{C}}{\overset{\text{H}\quad\text{OH}}{}} & \text{CO}_2\text{Na}& \text{CO}_2\text{H}& \text{CO}\\
\text{MeOCH}& \text{MeOCH}& \text{MeOCH}& \text{MeOCH}\\
\text{MeOCH}& \rightarrow\ \text{MeOCH}& \rightarrow\ \text{MeOCH}& \rightleftarrows\ \text{MeOCH}\\
\text{HCOMe}& \text{HCOMe}& \text{HCOMe}& \text{HCOMe}\\
\text{HCO}& \text{HCOH}& \text{HCOH}& \text{HCO}\\
\text{CH}_2\text{OMe}& \text{CH}_2\text{OMe}& \text{CH}_2\text{OMe}& \text{CH}_2\text{OMe}\\
n\text{-Tetramethylmannose}& & & \text{II}
\end{array}
$$

There had now been obtained by Haworth and by Irvine two series of methylated reducing sugars. The first or normal forms were those obtained from the ordinary methylglycosides, and the oxidation evidence gradually accumulated to show that all had the pyranose structure. The other series of methylated sugars was the one obtained from the Fischer γ-methylglycosides and contained the furanose (1,4-) ring. When the methylated sugars of either series were oxidized, the lactones of the corresponding methylated aldonic acids could be obtained. Of the two tetramethylgluconolactones, the γ- (1,4-) is crystalline and the δ- (1,5-) forms a crystalline phenylhydrazide. Their structures have also been determined by Haworth, Hirst, and Miller [136] (1927) by nitric acid oxidation.

Haworth and his students [137] (1926) studied the rate of lactone hydrolysis exhibited by the methylated aldonolactones. This was the reverse process of the one studied by Levene and Simms. The results showed that the lactones obtained from the methylated aldoses of the

[135] Levene and Simms, ibid., 68, 737 (1926).
[136] Haworth, Hirst, and Miller, J. Chem. Soc., 2436 (1927).
[137] Charlton, Haworth, and Peat, ibid., 89 (1926).

$$
\begin{array}{ccc}
\text{Tetramethyl-}\delta\text{-gluconolactone} & \substack{\textit{l-Xylotrimethoxy-}\\ \textit{glutaric acid}} & \substack{\textit{(dextro)-Dimethoxy-}\\ \textit{succinic acid}} \\
\begin{array}{c} \text{CO} \\ | \\ \text{HCOMe} \\ | \\ \text{MeOCH} \\ | \\ \text{HCOMe} \\ | \\ \text{HCO} \\ | \\ \text{CH}_2\text{OMe} \end{array}
&
\begin{array}{c} \text{CO}_2\text{H} \\ | \\ \text{HCOMe} \\ | \\ \text{MeOCH} \\ | \\ \text{HCOMe} \\ | \\ \text{CO}_2\text{H} \end{array}
&
\begin{array}{c} \text{CO}_2\text{H} \\ | \\ \text{HCOMe} \\ | \\ \text{MeOCH} \\ | \\ \text{CO}_2\text{H} \end{array}
\end{array}
$$

$$
\begin{array}{c}
\text{CO} \\ | \\ \text{HCOMe} \\ | \\ \text{MeOCH} \\ | \\ \text{HCO} \\ | \\ \text{HCOMe} \\ | \\ \text{CH}_2\text{OMe}
\end{array}
$$

Tetramethyl-γ-gluconolactone
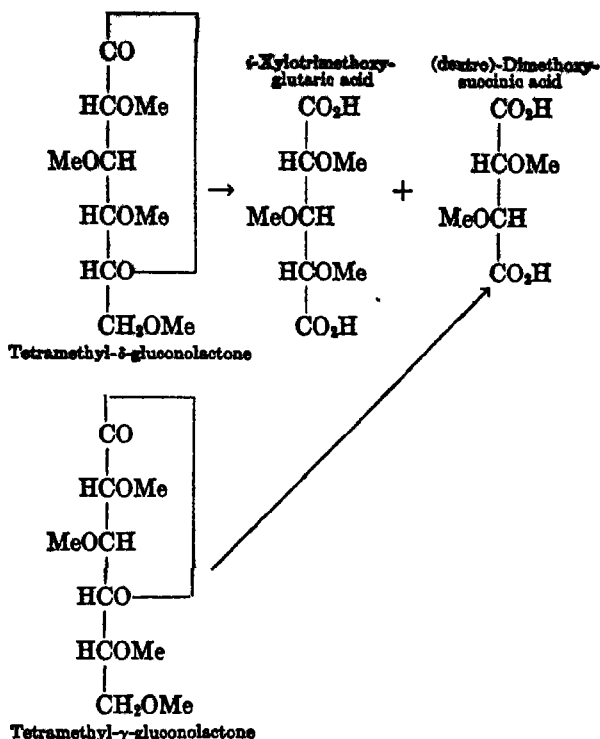
γ-methylglycoside series hydrolyzed very slowly and exhibited all the properties of γ-lactones. On the other hand, the (1,5-) or δ-lactones

I. Tetramethyl-*gamma*-galactonolactone
II. Trimethyl-*gamma*-arabonolactone
III. Tetramethyl-*gamma*-gluconolactone
IV. Tetramethyl-*delta*-gluconolactone
V. Tetramethyl-*delta*-galactonolactone
VI. Trimethyl-*delta*-arabonolactone
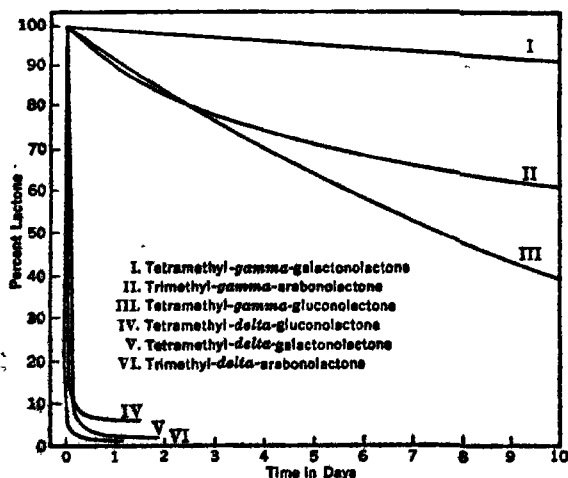
Fig. 2.*—Rates of hydrolysis of methylated lactones

obtained from the ordinary or normal methylglycoside series showed a high speed of hydrolysis. The behavior toward hydrolysis of the (1,4-) and (1,5-) oxygen rings in the methylated lactones of the aldonic acids is thus the reverse of that exhibited by the corresponding oxygen rings of like size when present in a methylglycoside structure.

It is inferred that the unstable lactones of the unmethylated aldonic acids noted by Nef and Hedenburg and by Levene and Simms are also δ-lactones. This inference is somewhat dangerous, as it involves reasoning by analogy. It is supported, however, by the optical data obtained by Levene and Simms for galactonic acid, in which a dextrorotatory lactone was formed first, followed by a levorotatory lactone. Now carbon atom five of d-galactonic acid is (+) and number four is (−), so that, if Hudson's rule may be extended to δ-lactones, the results are in harmony with a δ-structure.

d-Galactonic acid (dextro) (levo)
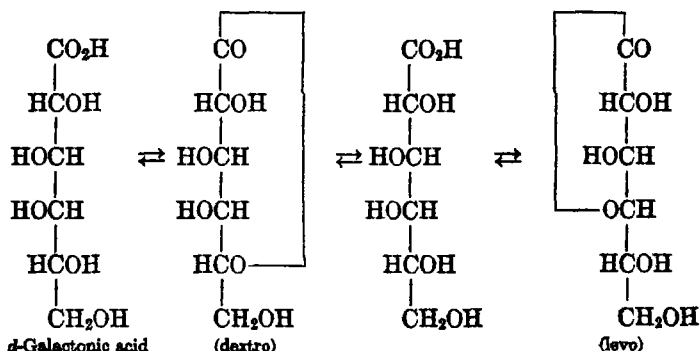
A further use has been made of the divergent properties of the two types of aldonolactones in the direct determination of aldose ring structure. The results of Armstrong (p. 1549) allowed the pyranose structure of the normal methylglucosides to be extended to glucose itself. This proof was practically unique for d-glucose because of the specificity of enzymic action. It was tacitly assumed that the pyranose structures obtained by methylation of the methylglycosides could be extended to the free sugars. This was somewhat obscured by the fact that the "normal" methylglycoside was the one produced by the apparently more vigorous methylglycosidic formation conditions, as has been noted. There was a need, then, for a more direct determination of the ring structures of the reducing sugars. This has been given by the studies initiated by Hudson and Isbell [138] (1932), and elaborated by Isbell, on the rapid oxidation of aldoses to aldonic acids by hypobromite. The results showed that there was an immediate formation of the δ-lactone,

[138] Isbell and Hudson, *Bur. Standards J. Research*, 8, 327 (1932).

and good proof was given that the free aldonic acid was not an intermediate. This procedure was also applicable to the available α- and β-forms of the aldoses, and significant differences in the rates of oxidation of the α- and β-isomers were noted. The data indicate that the ordinary crystalline forms of the aldose sugars possess pyranose structures and that the pyranose forms are the main components of the equilibrium system, although small amounts of other structures are not excluded.

$$
\begin{array}{ccc}
\overline{}\text{HCOH} & & \overline{}\text{C}{=}\text{O} \\
| & & | \\
(\text{CHOH})_3 & \rightarrow & (\text{CHOH})_3 \\
| & & | \\
\text{CHO}\underline{} & & \text{CHO}\underline{} \\
| & & | \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH}
\end{array}
$$
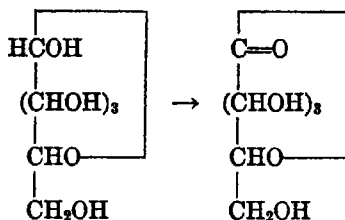
A very interesting result obtained by Isbell [139] (1933) was that a calcium chloride compound of mannose, isolated by Dale [140] (1929), which showed a peculiar and very rapid initial rotatory change in solution, produced a γ-lactone on hypobromite oxidation. Apparently this calcium chloride compound of mannose then possesses a furanose ring structure, and from this it may be concluded that mannofuranose was present in the aqueous solution from which the calcium chloride compound separated. All the above work on hypobromite oxidation rests on the premise that the unstable sugar lactones possess a δ-structure and is uncertain to the extent that this premise is uncertain.

Determination of Ring Structure by Means of the Glycol-Splitting Reagents. The remarkable discovery of Malaprade [141] (1928) that α-glycols undergo quantitative fission at room temperature with periodic acid or its salts opened new vistas in the general field of the degradative oxidation of organic compounds. The original interest of Malaprade was analytical in nature, and he cited his discovery as a method of analysis for the periodate ion in the presence of the iodate ion. In application to a polyhydric alcohol, Malaprade showed that the reaction took the following course:

$$
\text{CH}_2\text{OH}{-}(\text{CHOH})_x{-}\text{CH}_2\text{OH} + (n+1)\text{HIO}_4 \rightarrow
$$
$$
(n+1)\text{HIO}_3 + \text{H}_2\text{O} + 2\text{HCHO} + n\text{HCOOH}
$$
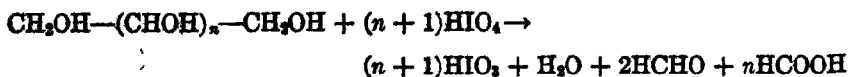
Hérissey, Fleury, and Joly [142] (1934) correctly interpreted the analyti-

---

[139] Isbell, *J. Am. Chem. Soc.*, **55**, 2166 (1933).

[140] Dale, *ibid.*, **51**, 2788 (1929).

[141] Malaprade, *Bull. soc. chim.*, [4] **43**, 683 (1928); *ibid.*, [5] **1**, 833 (1934).

[142] Hérissey, Fleury, and Joly, *J. pharm. chim.*, [8] **20**, 149 (1934).

cal data obtained when periodic acid reacted with an aldohexopyrano-
side.

$$CH_2OH\text{---}\overset{\overset{\displaystyle H}{|}}{CH}\text{---}(CHOH)_3\text{---}\underset{\underset{\displaystyle O\text{---------------}}{|}}{C}OMe + 2HIO_4 \rightarrow$$

$$2HIO_3 + H_2O + \overset{\overset{\displaystyle CHO}{|}}{\underset{\underset{\displaystyle CH_2OH}{|}}{CH}}\text{------}O\text{------}\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle CHO}{|}}{C}OMe} + HCOOH$$
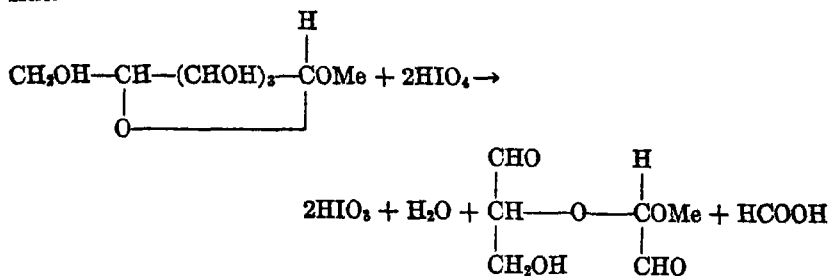
The isolation and characterization of the reaction product were
effected by Jackson and Hudson [143] (1936) for the case of β-methyl-d-
glucopyranoside as follows:

$$\overset{\overset{\displaystyle CHO}{|}}{\underset{\underset{\displaystyle CH_2OH}{|}}{CH}}\text{------}O\text{------}\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle CHO}{|}}{C}OMe} \xrightarrow{Ba(OBr)_2} Ba \overset{...}{\underset{...}{\begin{array}{c}CH_2O\;\;\;H\\ C\\ O\text{---}C=O\\ O\text{---}C=O\\ HCO\\ CH_2OH\end{array}}} \xrightarrow{H_2O+[O]} \overset{\overset{\displaystyle CO_2H}{|}}{\underset{\underset{\displaystyle CO_2H}{|}}{}} + \overset{\overset{\displaystyle CO_2H}{|}}{\underset{\underset{\displaystyle CH_2OH}{|}}{HCOH}}$$
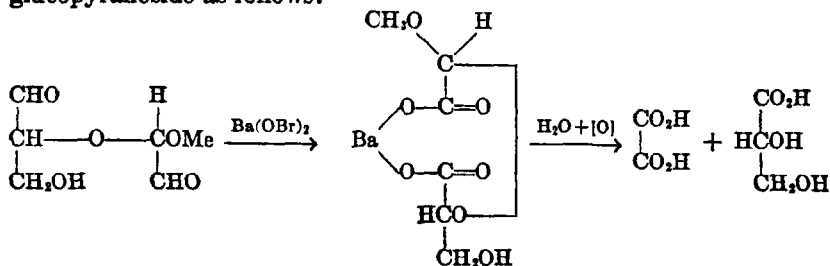
The intermediate barium salt of the substance termed D'-methoxy-D-
hydroxymethyl-diglycolic acid was isolated in crystalline form, and the
d(levo)-glyceric acid was isolated as its crystalline calcium salt. Defini-
tive differences can be predicted for a furanoside ring and such predic-
tions were verified by Jackson and Hudson [143] for crystalline α-methyl-
d-arabinofuranoside. In this case the optically active dibasic acid ob-
tained is identical with that from an aldo-d-hexopyranoside and formic
acid is not produced.

Another α-glycol-splitting reagent is lead tetraacetate, discovered by
Criegee [144] (1930). The action of this reagent on polyhydric alcohols
and glycosides is identical with that of periodic acid [145] and with certain
restrictions is applicable in aqueous solution.[146] For preparative work
the lead tetraacetate procedure has certain advantages. These new
methods for the determination of ring structure offer great simplifications
over the laborious methylation techniques previously used.

[143] Jackson and Hudson, J. Am. Chem. Soc., 58, 378 (1936); ibid., 59, 994 (1937).
[144] Criegee, Ann., 481, 275 (1930); Ber., 64, 260 (1931); Angew. Chem., 50, 153 (1937).
[145] Criegee, Ann., 495, 211 (1932); Karrer and Hirohata, Helv. Chim. Acta, 16, 959 (1933); McClenahan and Hockett, J. Am. Chem. Soc., 60, 2061 (1938).
[146] Baer, Grosheints, and H. O. L. Fischer· J. Am. Chem. Soc., 61, 2607 (1939).

Configuration of the Reducing Carbon Atom. It has been noted previously that to obtain a complete solution for the structure of a cyclic sugar or its derivative it is necessary to determine the relative configuration of the reducing carbon (for an aldose, carbon one) in addition to determining the point of ring closure. The method of α-,β-designation initiated by Hudson (p. 1550) is empirical and bears no necessary relation to true relative configuration.

Böeseken [147] (1913) made an attempt to solve this question of the space position of the groups attached to carbon one of d-glucose. This was based upon his observations regarding the effect of the constitution of hydroxy compounds on the electrical conductivity of boric acid solutions—namely, that a *cis* configuration in a cyclic glycol produced a complex with boric acid which was a stronger acid than that produced by the *trans* isomer. The conductivity of α-d-glucose in the presence of boric acid decreases during mutarotation as it is converted in part into β-d-glucose; the reverse is true of β-d-glucose. The velocity of this change parallels that of the mutarotation. On this basis, α-d-glucose may be assigned the formula:

$$
\begin{array}{l}
\text{HCOH} \\
\text{HCOH} \\
\text{HOCH} \\
\text{HCOH} \\
\text{HCO}\!-\!\!\rule{0pt}{0pt} \\
\text{CH}_2\text{OH}
\end{array}
$$

If the above establishment of structure on the basis of physical measurements be accepted, the configuration of carbon one of d-glucose is correlated to the Rosanoff classification. Then, if the Armstrong correlation of α-d-glucose with α-methyl-d-glucopyranoside (p. 1549) be accepted, the methoxyl can be written to the right in the projection formula of the latter substance.

$$
\begin{array}{l}
\text{HC}\!-\!\text{OMe} \\
\text{HCOH}
\end{array}
$$
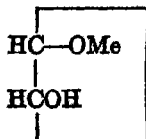
The procedure of Jackson and Hudson,[148] previously discussed (p. 1569) as a method for ascertaining the point of ring closure, at the same time offers a method for the correlation of the configuration of

[147] Böeseken, *Ber.*, **46**, 2612 (1913).

carbon one. When a methyl aldopentopyranoside is subjected to periodic acid oxidation followed by hypobromite oxidation, the dibasic acid (isolated as the crystalline strontium salt) produced has the following structure:

$$
\begin{array}{c}
\text{OCH}_3 \\
| \\
\text{H—C—O—CH}_2\text{—CO}_2\text{H} \\
| \\
\text{CO}_2\text{H}
\end{array}
$$
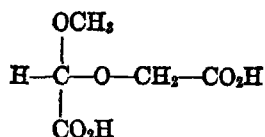
The only asymmetric center left in the above substance is that of carbon one. With a methyl aldo-*d*-hexopyranoside, the product contains two asymmetric centers: one, as above; and the other, the asymmetric carbon that was originally carbon five and is thus of identical configuration (+) for all members of the *d*-series of aldohexopyranosides.

$$
\begin{array}{cc}
\text{OCH}_3 & \text{CH}_2\text{OH} \\
| & | \\
\text{H—C} \quad\text{—O—}\quad \text{C—H} \\
| & | \\
\text{CO}_2\text{H} & \text{CO}_2\text{H}
\end{array}
$$
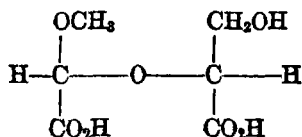
It is obvious that a direct configurational correlation, based upon the order of the groups about carbon one, may thus be made. The results of these important researches are tabulated in Table III. So far, the

TABLE III

Glycoside	$[\alpha]_D^{20}$ (water)	
	Glycoside	Dibasic Acid
α-Methyl-*d*-arabinopyranoside	−17°	−12.7°
α-Methyl-*d*-xylopyranoside	+154	−12.1
α-Methyl-*d*-lyxopyranoside [143]	+59	−11.5
β-Methyl-*d*-arabinopyranoside	−245	+12.5
β-Methyl-*d*-xylopyranoside	−65	+12.2
α-Methyl-*d*-glucopyranoside	+159	+26.0
α-Methyl-*d*-galactopyranoside	+196	+25.4
α-Methyl-*d*-mannopyranoside	+79	+26.3
α-Methyl-*d*-gulopyranoside	+120	+25.4
β-Methyl-*d*-glucopyranoside	−34	+45.0
β-Methyl-*d*-mannopyranoside [140]	−69	+45.4
β-Methyl-*d*-galactopyranoside [140]	+1	+45.0

[143] McClenahan and Hockett, *J. Am. Chem. Soc.*, **60**, 2061 (1938).
[140] Jackson and Hudson, *ibid.*, **61**, 959 (1939).

correlations have been consistent with the Hudson α-,β-empirical rule. The data of Table III show that, in the pentose series, the α(original Hudson assignment) -forms of the glycosides give the same dibasic acid as the final oxidation product and the β-forms give the enantiomorph. In the hexose series, the dibasic acids produced are diastereoisomeric but the same oxidation product is obtained from all the α-glycosides and a second oxidation product (dibasic acid) is produced from all the β-glycosides.

Naturally Occurring Glycosides and Their Synthesis. A wide variety of glycosides of alcohols and phenols are found in the plant world. On hydrolysis by acids or by enzymes, the glycosides produce one or more sugars, chiefly d-glucose, and the non-sugar portion, which is termed the aglucon. These aglucons are of a very diversified nature (pp. 1319 and 1427). It is remarkable that the naturally occurring glycosides are for the most part levorotatory and are hydrolyzable by emulsin. They accordingly belong to the β-glycosides. The α-glycosides are hydrolyzed in the main only by maltase. Helferich and his students have made very extensive studies of the hydrolysis of glycosides by the β-glucosidase of emulsin, and Bourquelot and co-workers [150] (1912) have synthesized β-glycosides by the reversal of the emulsin hydrolytic reaction.

The function of glycosides in the plant is rather obscure, but the physiological actions of many are well established, and it is to the presence of such glycosides that many herbs and roots owe their medicinal value. A few of the large number of naturally occurring glycosides are tabulated in Table IV.

Salicin is a classical example of a simple glycoside. It occurs in willow bark and has the following constitution:
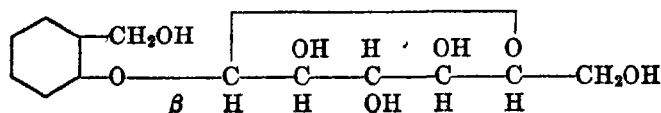
Irvine and Rose [151] (1906) methylated this substance to form a crystalline pentamethylsalicin which produced tetramethylglucopyranose on hydrolysis. Levene and Tipson [152] (1931) have found from methylation studies that some of the glycosides (nucleosides) produced by the partial hydrolysis of nucleic acids are furanosides.

The structures of a number of the naturally occurring glycosides have been verified by synthetic methods. The previously described method

[150] Bourquelot and Bridel, *Compt. rend.*, **155**, 86 (1912).

[151] Irvine and Rose, *J. Chem. Soc.*, **89**, 814 (1906).

[152] Levene and Tipson, *Science*, **74**, 521 (1931); *J. Biol. Chem.*, **94**, 809 (1932).

TABLE IV

REPRESENTATIVE GLYCOSIDES OF NATURAL OCCURRENCE

Glycoside	Sugar	Aglucon
Populin	Benzoylglucose	o-Hydroxybenzyl alcohol
Coniferin	Glucose	Coniferyl alcohol
Aesculin	Glucose	6,7-Dihydroxycoumarin
Idaein	Galactose	Cyanidin (p. 1318)
Scopolin	Glucose (2 moles)	6-Methylaesculetin
Peonin	Glucose (2 moles)	Cyanidin
Violanin	Rhamnose + glucose	Delphinidin (p. 1319)
Digitoxin	Digitoxose (3 moles)	Digitoxigenin (p. 1443)
Cymarin	Cymarose	Strophanthidin (p. 1435)
Prunasin	Glucose	Mandelonitrile
Lotusin	Gentiobiose	Lotoflavin
Hesperidin	Glucose + rhamnose	Hesperitin
Salicin	Glucose	o-Hydroxybenzyl alcohol

of Fischer produced the isomeric glycosides directly from the sugar and alcohol. The other and more selective method is that employing the acetohalogen sugars as condensing agents.

The first acetohalogen sugar, acetochloroglucose, was prepared by Colley [153] (1870) by the reaction between glucose and five moles of acetyl chloride. It is now known that this substance has the structure

$$CH_2OAc-CH-(CHOAc)_3-\overset{\overset{\displaystyle H}{|}}{\underset{\underline{\hspace{1cm}O\hspace{1cm}}}{C}}-Cl.$$ A. Michael [154] (1879) reacted this
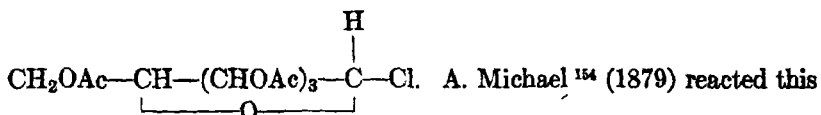
compound with potassium phenolate and obtained crystalline β-phenyl-glucoside. In an analogous manner, Michael [155] (1881) performed the first artificial synthesis of a naturally occurring glycoside and obtained

methyl arbutin, CH_3O—⟨ ⟩—$OC_6H_{11}O_5$. A more reactive and
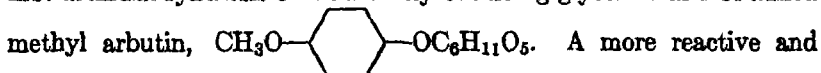
useful compound than acetochloroglucose was obtained when Koenigs and Knorr [156] (1901) prepared crystalline acetobromoglucose by the action of acetyl bromide upon glucose. These workers showed that this compound reacted readily with methanol in the presence of silver carbonate to form β-methylglucoside tetraacetate. Koenigs and Knorr

[153] Colley, Ann. chim. phys., [4] 21, 363 (1870).
[154] Michael, Am. Chem. J., 1, 305 (1879).
[155] Michael, Ber., 14, 2097 (1881).
[156] Koenigs and Knorr, Ber., 34, 973 (1901).

also showed that the crystalline acetonitroglucose, obtained by Colley [157] (1873), reacted in a similar manner with methanol in the presence of barium carbonate to form the tetraacetate of β-methylglucoside. Aceto-nitroglucose has the structure $CH_2OAcCH—(CHOAc)_3—CHONO_2$.

$$\overline{\qquad\qquad O \qquad\qquad}$$

Deacetylation of the above glucoside tetraacetate produced the previously known β-methylglucoside. As this substance was later shown to be a pyranoside, it may be concluded that the glucosides synthesized from acetobromoglucose possess the pyranose ring structure and the β-configuration, although some exceptions to the latter are known.[158]
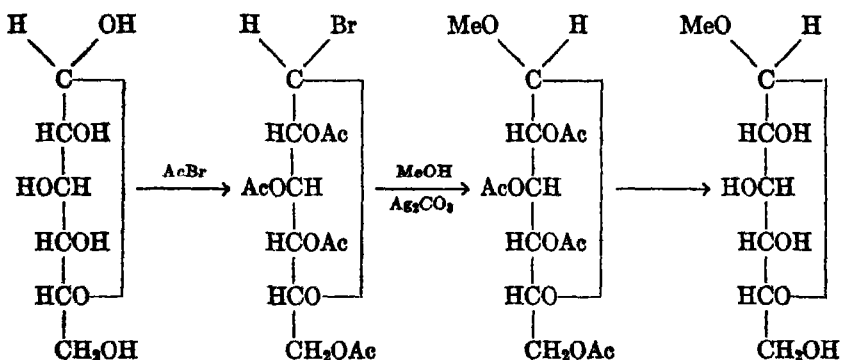
E. Fischer [159] (1911) improved the method for preparing the aceto-halogen sugars by employing the reaction between the hexose penta-acetate (either α- or β-) and a glacial acetic acid solution of the halogen acid. In this manner, acetohalogen sugars containing chlorine, bromine, or iodine were obtained, and in 1923 Brauns [160] prepared acetofluoroglu-cose. Brauns has extended these reactions to many of the reducing sugars and has measured the optical rotations of the acetohalogen sugars with high precision. He has deduced a relationship between these rota-tion values and the atomic dimensions of the halogens.[161]

The halogen of these acetohalogen sugars may be replaced by hy-droxyl on controlled hydrolysis to form the mutarotatory hexose tetra-acetate [162] (Fischer and Delbrück, 1909). Ethylation by the Purdie reaction of such a tetraacetate, obtained [163] (Hudson and Johnson, 1916) from the third form of galactose pentaacetate, produced on deacetylation

[157] Colley, *Compt. rend.*, **76**, 436 (1873).
[158] Levene and Wolfrom, *J. Biol. Chem.*, **78**, 525 (1928).
[159] Fischer, *Ber.*, **44**, 1899 (1911).
[160] Brauns, *J. Am. Chem. Soc.*, **45**, 833 (1923).
[161] Brauns, *Bur. Standards J. Research*, **7**, 573 (1931); summarizing paper.
[162] Fischer and Delbrück, *Ber.*, **42**, 2776 (1909).
[163] Hudson and Johnson, *J. Am. Chem. Soc.*, **38**, 1223 (1916).

a new crystalline ethylgalactoside of a non-pyranose structure [164] (Schlubach and Meisenheimer, 1934).

Zemplén [165] (1929) has solved the problem of obtaining the isomeric α-glycosides through the Koenigs and Knorr reaction by the substitution of mercuric acetate for the silver salt. Pacsu [166] (1928) was able to convert β-methylglucoside tetraacetate quantitatively into the α-form by heating with titanium tetrachloride in chloroform solution. Helferich and co-workers [167] (1933) have made the phenolic α-glycosides available by the procedure of fusing the sugar acetate with the phenol and zinc chloride.

Acyclic Sugar Structures. It has been seen how the original aldehyde formula for d-glucose gave way to the lactol or cyclic hemiacetal structure required to explain further isomerism. It became a matter of considerable interest, then, when in 1926 Levene and Meyer [168] obtained a pentamethylglucose which contained no ring in its structure. This was synthesized from glucose ethyl mercaptal. Although Fischer was unable to prepare an acetal of glucose, he succeeded in preparing a thioacetal or mercaptal by reacting glucose with ethyl mercaptan in concentrated hydrochloric acid solution [169] (1894). Levene and Meyer methylated this crystalline substance and removed the thioacetal groups with mercuric chloride and water, obtaining the product as a syrup. This reaction was extended to galactose and mannose [170] (1927).

In 1929 Wolfrom [171] obtained a crystalline open chain or *aldehydo*-pentaacetate of d-glucose similar in structure to the above. The method used was a hydrolysis of the acetylated glucose ethyl mercaptal in dilute acetone solution by reaction with mercuric chloride in the presence of cadmium carbonate.

$$\begin{array}{ccc} HC\begin{array}{l}SEt\\ \\SEt\end{array} & & CHO \\ | & \xrightarrow[HgCl_2+H_2O]{CdCO_3} & | \\ (CHOAc)_4 & & (CHOAc)_4 + 2ClHgSEt + CdCl_2 + CO_2 \\ | & & | \\ CH_2OAc & & CH_2OAc \end{array}$$
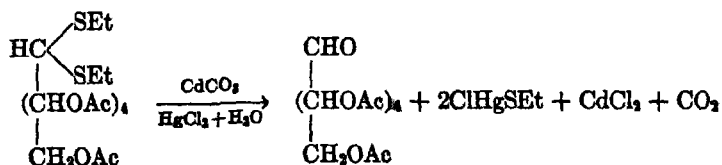
The substance readily formed a semicarbazone without loss of an acetate group and gave a Schiff aldehyde test. The reaction was later ex-

[164] Schlubach and Meisenheimer, Ber., 67, 429 (1934).
[165] Zemplén, Ber., 62, 990 (1929); Zemplén and Gerecs, Ber., 63, 2720 (1930).
[166] Pacsu, Ber., 61, 137, 1513 (1928).
[167] Helferich and Schmitz-Hillebrecht, Ber., 66, 378 (1933).
[168] Levene and Meyer, J. Biol. Chem., 69, 175 (1926).
[169] Fischer, Ber., 27, 673 (1894).
[170] Levene and Meyer, J. Biol. Chem., 74, 695 (1927).
[171] Wolfrom, J. Am. Chem. Soc., 51, 2188 (1929).

tended to several other sugar structures. *aldehydo*-Glucose pentaacetate has been prepared by the catalytic reduction of gluconyl chloride penta-acetate [172] (1936), obtained from gluconic acid pentaacetate. A few of the fully acetylated and non-lactonized sugar acids are obtainable by direct acetylation methods.[173] Hurd and Sowden [174] (1938) devised a general procedure for their synthesis by the deamination of the acety-lated aldonamides.

The synthesis of *aldehydo*-galactose pentaacetate [175] (1930) added a fifth crystalline pentaacetate to the four then known. This latter sub-stance also formed crystalline carbonyl addition compounds with alcohols and water which by their distinctive rotations were shown to be true val-ence compounds. In 1930 Brigl and Mühlschlegel[176] obtained an *aldehydo*-pentabenzoate of glucose which crystallized as an alcohol addition com-pound, apparently an ethyl hemiacetal or carbonyl addition compound. This would indicate that the nature of the substituent groups apparently influences the stability of the carbonyl group, as such addition com-pounds did not form with the acetate, although the mutarotation exhibited by the acetate in alcohol showed the formation of such struc-tures in solution. The mutarotation exhibited by an *aldehydo*-acetate in alcohol can be explained by carbon one becoming asymmetric through hemiacetal formation. Such a pair of isomers has been isolated for the ethyl hemiacetals of methyl *aldehydo-d*-galacturonate (p. 1590) tetra-acetate by Dimler and Link [177] (1940), who designated the isomers by the prefixes α and β. To prevent confusion with the usual α,-β-cyclic sugar nomenclature, the prefix *aldehydo* is included in the name.

$$
\begin{matrix}
\text{OH} & & & & \text{OCH}_3 \\
| & & & & | \\
\text{HCOCH}_3 & \rightleftarrows \text{HC=O} & + \text{MeOH} & \rightleftarrows & \text{HCOH} \\
| & | & & & | \\
(\text{CHOAc})_4 & (\text{CHOAc})_4 & & & (\text{CHOAc})_4 \\
| & | & & & | \\
\text{CH}_2\text{OAc} & \text{CH}_2\text{OAc} & & & \text{CH}_2\text{OAc} \\
\text{I} & \text{II} & & & \text{I}'
\end{matrix}
$$
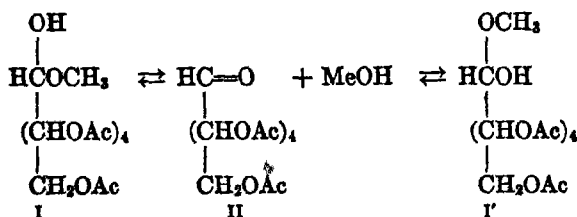
A typical sugar mutarotation curve is obtained when an *aldehydo*-acetate (II) is dissolved in methanol, and a similar type was found by Wolfrom and Morgan [178] (1932) for *aldehydo*-galactose pentaacetate

[172] Cook and Major, *ibid.*, **58**, 2410 (1936).
[173] Major and Cook, *ibid.*, **58**, 2574 (1936); Robbins and Upson, *ibid.*, **62**, 1074 (1940).
[174] Hurd and Sowden, *ibid.*, **60**, 235 (1938).
[175] Wolfrom, *ibid.*, **52**, 2464 (1930).
[176] Brigl and Mühlschlegel, *Ber.*, **63**, 1551 (1930).
[177] Dimler and Link, *J. Am. Chem. Soc.*, **62**, 1216 (1940).
[178] Wolfrom and Morgan, *ibid.*, **54**, 3390 (1932).

methyl hemiacetal (I) in methanol solution. The three-membered nature of the *aldehydo*-acetate and methanol equilibrium can be demonstrated by the complex nature of the mutarotation curve obtained when I is dissolved in pure chloroform. The free carbonyl form (II) of an *aldehydo*-acetate shows no mutarotation in pure chloroform. Galactose pentaacetate aldehydrol exhibits no mutarotation in water, carbon one not being asymmetric; but in chloroform solution the water dissociates from the carbonyl and a monomolecular decomposition curve results.

$$HC \underset{\textstyle \overset{|}{(CHOAc)_4}}{<} \overset{\textstyle OH}{\underset{\textstyle OH}{}} \quad \rightleftarrows \quad HC{=}O$$

$$\begin{array}{cc} HC{\Large<}^{OH}_{OH} & HC{=}O \\ | & | \\ (CHOAc)_4 & \rightleftarrows \quad (CHOAc)_4 + H_2O \\ | & | \\ CH_2OAc & CH_2OAc \end{array}$$
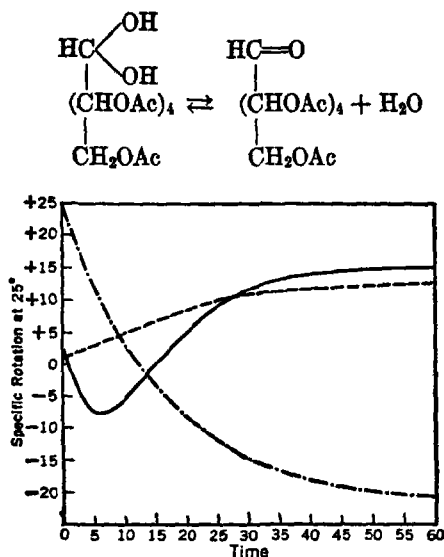
FIG. 3.—Mutarotation characteristics of *aldehydo*-sugar acetates. ——, *aldehydo-d*-galactose methyl hemiacetal in chloroform (alcohol-free), time in hours; – – – –, *aldehydo-d*-galactose methyl hemiacetal in methanol, time in hours; —·—·—·—, *aldehydo-d*-galactose aldehydrol in chloroform (alcohol-free), time in minutes.

Micheel [179] (1935) and Wolfrom [180] (1935) prepared fully acetylated aldose sugars in which the carbonyl group was in the diacetate form. Pirie [181] (1936) obtained these structures by the acetylation of sugar mercaptals with acetic anhydride and sulfuric acid and isolated *d,l*-galactose heptaacetate from agar by the use of these reagents. An acyclic acetohalogen galactose [182] has been reported, and the similar structure in the arabinose series has been found by Felton and W.

[179] Micheel, Ruhkopf, and Suckfüll, *Ber.*, **68**, 1523 (1935).
[180] Wolfrom, *J. Am. Chem. Soc.*, **57**, 2498 (1935).
[181] Pirie, *Biochem. J.*, **30**, 374 (1936).
[182] Wolfrom, *J. Am. Chem. Soc.*, **57**, 2498 (1935).

Freudenberg [163] (1935) as a by-product in the preparation of the cyclic form of acetobromoarabinose. 1,1-Dichloro-*aldehydo*-galactose pentaacetate has been isolated [184] (1940).

$$
\begin{array}{cc}
\text{HC}\diagup^{\text{OAc}}_{\text{OAc}} & \text{HC}\diagup^{\text{OAc}}_{\text{Br}} \\
| & | \\
(\text{CHOAc})_4 & (\text{CHOAc})_4 \\
| & | \\
\text{CH}_2\text{OAc} & \text{CH}_2\text{OAc}
\end{array}
$$

Hudson and co-workers [185] (1937) subjected the methylarabinopyranosides (I) to acetolysis, and with this sugar structure they obtained the acyclic derivative II in two forms. These forms differ only by the asymmetry of carbon one and are interconvertible by acetic anhydride and zinc chloride.

$$
\begin{array}{ccccc}
\text{H}\diagdown\quad\diagup\text{OMe} & & \text{OMe} & & \text{OMe} \\
\text{C}\!-\!-\!- & & | & & | \\
| & & \text{H}\!-\!\text{C}\!-\!\text{OAc} & & \text{H}\!-\!\text{C}\!-\!\text{Cl} \\
\text{HOCH} & & | & & | \\
| & \rightarrow & (\text{CHOAc})_3 & \rightarrow & (\text{CHOAc})_3 \\
\text{HCOH} & & | & & | \\
| & & \text{CH}_2\text{OAc} & & \text{CH}_2\text{OAc} \\
\text{HCOH} & & \text{II} & & \text{III} \\
| & & & & \\
\text{CH}_2\text{O}\!-\!\! & & & & \\
\text{I} & & \text{OMe} & & \text{OMe} \\
& & | & & | \\
& & \text{H}\!-\!\text{C}\!-\!\text{OMe} & & \text{H}\!-\!\text{C}\!-\!\text{OMe} \\
& & | & & | \\
& & (\text{CHOH})_3 & \leftarrow & (\text{CHOAc})_3 \quad\leftarrow \\
& & | & & | \\
& & \text{CH}_2\text{OH} & & \text{CH}_2\text{OAc} \\
& & \text{V} & & \text{IV}
\end{array}
$$
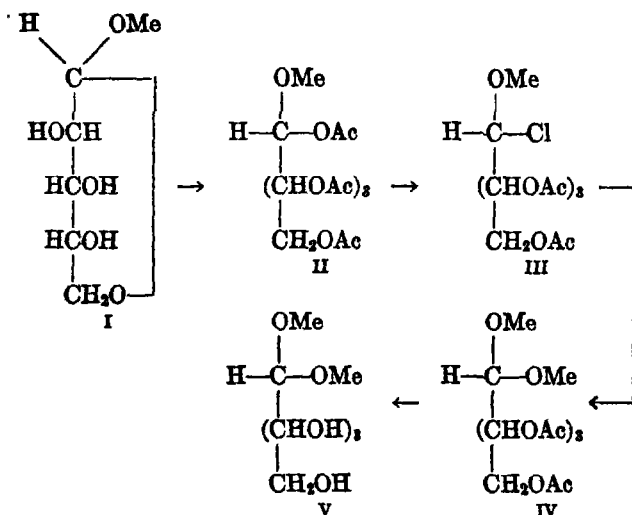
Replacement of the 1-acetate in II by chlorine led to III, also isolated in two forms, which in turn led to the synthesis of the crystalline dimethyl acetal of *d*-arabinose (V). Sugar derivatives of types II and III also have been obtained by direct operations on the *aldehydo*-sugar acetates, and in some cases isomeric forms of type II have been isolated.[186]

A general synthesis for the sugar acetals is that involving demercaptalation of the acetylated thioacetals in the presence of an alcohol, with subsequent saponification of the acetate groups. This was first applied

[163] Felton and W. Freudenberg, *ibid.*, **57**, 1637 (1935).

[184] Wolfrom and Weisblat, *ibid.*, **62**, 1149 (1940).

[185] Montgomery, Hann, and Hudson, *ibid.*, **59**, 1124 (1937).

[186] Wolfrom and Konigsberg, *ibid.*, **60**, 288 (1938); Wolfrom, Konigsberg, and Moody *ibid.*, **62**, 2343 (1940).

to galactose [187] (1938) and successfully extended to glucose [188] (Wolfrom and Waisbrot, 1938) and to fructose [189] (Pacsu, 1939), whose thioacetal was available by an indirect method [190] (1934). A mixed oxygen-sulfur acetal of glucose has been reported.[191]

A striking result was obtained by Pacsu and Rich [192] (1932), when they demonstrated that an open-chain or *keto*-structure was present in a pentaacetate of fructose prepared long before by Hudson and Brauns [193] (1915) by direct acetylation methods. A similar finding was made by Montgomery and Hudson [194] (1934), when they discovered an *aldehydo*-structure in a hexaacetate of d-[α-mannoheptose] obtained by acetylation of the free sugar. *keto*-Fructose pentaacetate exhibits the properties of a hindered ketone, the carbonyl group being very unreactive.

Solutions of the lactol forms of the reducing sugars show the presence of their potential carbonyl group by forming typical amino condensation products, such as phenylhydrazones, oximes, and semicarbazones. For these, however, two types of structure are theoretically possible.

$$HC\!\!=\!\!NOH$$
$$|$$
$$(CHOH)_4$$
$$|$$
$$CH_2OH$$

$$H\diagdown \diagup NHOH$$
$$C$$
$$|$$
$$(CHOH)_3$$
$$|$$
$$CHO\!\!-\!\!$$
$$|$$
$$CH_2OH$$
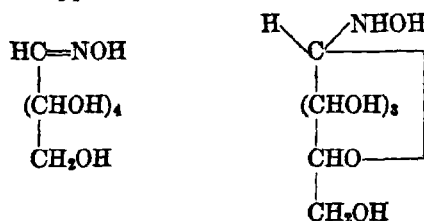
When a sugar amino condensation product is acetylated, the acetate obtained may be of either type, and frequently a mixture of both is produced. Glucose phenylhydrazone exists in two forms, one of which was shown by Behrend and Reinsberg [195] (1910) to produce on acetylation a pentaacetate of the ring-structure type.

$$H\diagdown \diagup NHN(Ac)C_6H_5$$
$$C$$
$$|$$
$$(CHOAc)_3$$
$$|$$
$$CHO\!\!-\!\!$$
$$|$$
$$CH_2OAc$$
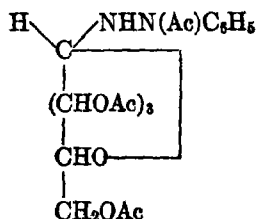
[187] Wolfrom, Tanghe, George, and Waisbrot, *ibid.*, **60**, 132 (1938); Campbell and Link, *J. Biol. Chem.*, **122**, 635 (1938).

[188] Wolfrom and Waisbrot, *J. Am. Chem. Soc.*, **60**, 854 (1938).

[189] Pacsu, *ibid.*, **61**, 1671 (1939).

[190] Wolfrom and Thompson, *ibid.*, **56**, 880 (1934).

[191] Wolfrom, Weisblat, and Hanze, *ibid.*, **62**, 3246 (1940).

[192] Pacsu and Rich, *ibid.*, **54**, 1697 (1932).

[193] Hudson and Brauns, *ibid.*, **37**, 1283 (1915).

[194] Montgomery and Hudson, *ibid.*, **56**, 2463 (1934).

[195] Behrend and Reinsberg, *Ann.*, **377**, 189 (1910).

Behrend isolated α-acetylphenylhydrazine on hydrolysis of this acetate. He thus allocated one of the five acetate groups in the acetylated hydrazone to the nitrogen and furnished excellent proof for a ring structure. Had the structure been acyclic, a hexaacetate would have been indicated. This principle of establishing ring structures by detecting the presence of an N-acetyl group can be effected readily by analytical methods that distinguish between N-acetyl and O-acetyl.[196] The same result can be obtained by comparing the acetylated nitrogen compound with that obtained directly by reaction of the *aldehydo*-acetate with the amino reagent.

$$HC\!\!=\!\!NNHC_6H_5 \qquad HC\!\!=\!\!NNHC_6H_5 \qquad O\!\!=\!\!CH$$
$$(CHOH)_4 \xrightarrow[\text{Pyridine}]{Ac_2O} (CHOAc)_4 \xleftarrow{C_6H_5NHNH_2} (CHOAc)_4$$
$$CH_2OH \qquad CH_2OAc \qquad CH_2OAc$$
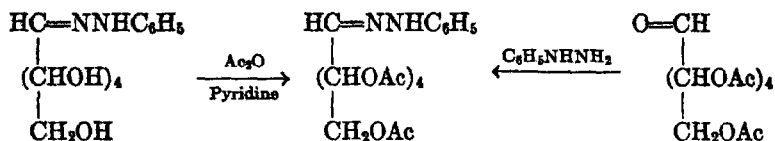
By these methods the structures of a number of acetylated sugar amino condensation products have been demonstrated.[197, 198] Thus, glucose (and galactose) phenylosazone tetraacetate is acyclic; galactose phenylhydrazone pentaacetate is acyclic; and both the acyclic (*aldehydo*) and ring types of acetylated oximes and semicarbazones have been found. When the acetylated oxime or semicarbazone is acyclic, the *aldehydo*-form of the sugar acetate may be obtained by treatment with nitrous acid (Wolfrom and Georges,[199] 1934).

$$HC\!\!=\!\!NNHCONH_2 \qquad HC\!\!=\!\!O$$
$$(CHOAc)_4 \xrightarrow{HNO_2} (CHOAc)_4$$
$$CH_2OAc \qquad CH_2OAc$$

$$HC\!\!=\!\!NOAc \qquad HC\!\!=\!\!NOH$$
$$(CHOAc)_4 \xrightarrow{(CO_2H)_2} (CHOAc)_4 \xrightarrow{HNO_2}$$
$$CH_2OAc \qquad CH_2OAc$$
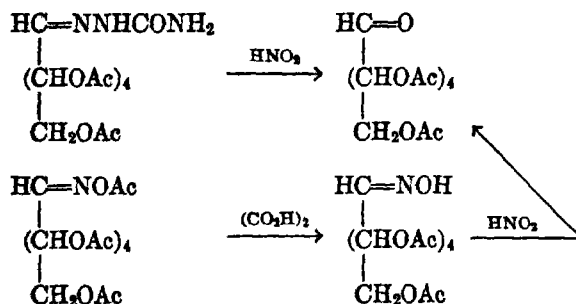
It is apparent from the preceding exposition that the acyclic structure of the sugars is well established in crystalline derivatives, many of which have been obtained directly from solutions of the sugars and thus offer evidence that these acyclic structures were present in such solutions. It

[196] Wolfrom, Konigsberg, and Soltzberg, *J. Am. Chem. Soc.*, **58**, 490 (1936).

[197] Wolfrom and Thompson, *ibid.*, **53**, 622 (1931); Wolfrom and Christman, *ibid.*, **53**, 3413 (1931); Wolfrom, Georges, and Soltzberg, *ibid.*, **56**, 1794 (1934); **58**, 1781, 1783 (1936).

[198] Deulofeu, Wolfrom, Cattaneo, Christman, and Georges, *ibid.*, **55**, 3488 (1933), Deulofeu, Cattaneo, and Mendivelzua, *J. Chem. Soc.*, 147 (1934); Restelli de Labriola and Deulofeu, *J. Am. Chem. Soc.*, **62**, 1611 (1940).

[199] Wolfrom and Georges, *J. Am. Chem. Soc.*, **56**, 1794 (1934).

has also been noted that the mutarotation of the sugars is perhaps best explained by the assumption of an intermediate acyclic form (p. 1549). Marchlewski [200] (1933) has shown that a carbonyl absorption band appears immediately on the addition of alkali to glucose and other reducing sugars and disappears on neutralization of the alkali. It is also well known that a slight alkalinity is essential for the cyanohydrin reaction, in which the acyclic form of the sugar is the logical intermediate. Supporting evidence for such an intermediate is furnished by measurements of the initial hydrogen cyanide binding capacity of sugars [201, 202] (Lippich, 1932; Brigl, 1931), and certain polarographic measurements have been interpreted to indicate the presence of small amounts of the *aldehydo*-form in aqueous solutions of glucose and other sugars [203] (1940).

Other Ring Structures. It is of great interest to note that Brigl and co-workers [204] (1931) obtained a tetrabenzoate of glucose with the second position open. This substance showed no tendency to form a lactol or ethylene oxide ring, but showed all the properties of a true open-chain α-hydroxy aldehyde, reacting with diazomethane to form a methyl ketone. This eliminates the ethylene oxide ring as a possibility with glucose, at least in its benzoate structure. The reactions employed by Brigl are diagrammed below.

$$
\begin{array}{ccccccc}
\text{HC(SEt)}_2 & \text{HC(SEt)}_2 & \text{HC=O} & \xrightarrow{\text{CH}_2\text{N}_2} & \text{CH}_3 \\
| & | & | & & | \\
(\text{CHOH})_4 \rightarrow & \text{CHOH} \rightarrow & \text{CHOH} & & \text{C=O} \\
| & | & | & & | \\
\text{CH}_2\text{OH} & (\text{CHOBz})_3 & (\text{CHOBz})_3 & & \text{CHOH} \\
& | & | & & | \\
& \text{CH}_2\text{OBz} & \text{CH}_2\text{OBz} & & (\text{CHOBz})_3 \\
& & & & | \\
& & & & \text{CH}_2\text{OBz}
\end{array}
$$

$$
\begin{array}{ccccc}
\text{HC(SEt)}_2 & \text{CHOH} & & \text{HC=NNHC}_6\text{H}_5 \\
| & | & & | \\
\text{CHOMe} & \text{CHOMe} & & \text{C=NNHC}_6\text{H}_5 \\
| \quad \rightarrow & | & \rightarrow & | \\
(\text{CHOBz})_3 & \text{CHOH} & & (\text{CHOH})_3 \\
| & | & & | \\
\text{CH}_2\text{OBz} & \text{CHOH} & & \text{CH}_2\text{OH} \\
& | \\
& \text{CHO} \\
& | \\
& \text{CH}_2\text{OH}
\end{array}
$$
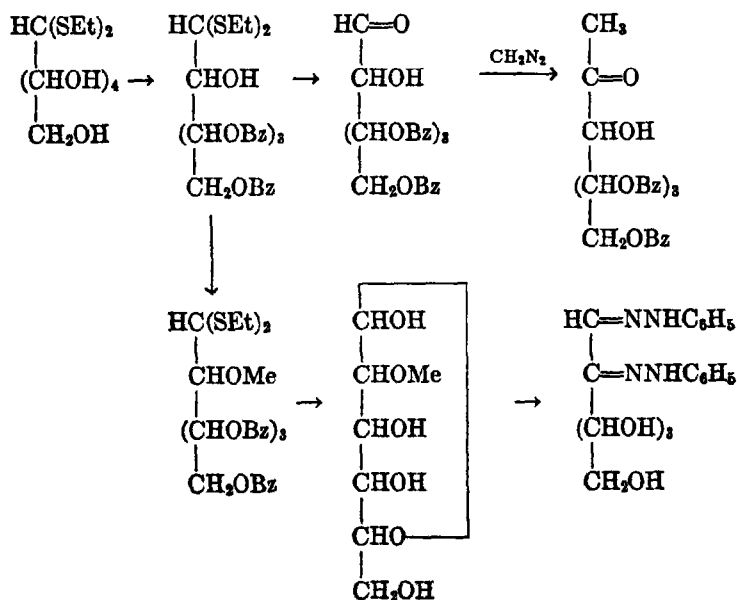
[200] Gabryelski and Marchlewski, *Biochem. Z.*, **261**, 393 (1933)
[201] Lippich, *ibid.*, **248**, 280 (1932).
[202] Brigl, Mühlschlegel, and Schinle, *Ber.*, **64**, 2921 (1931).
[203] Cantor and Peniston, *J. Am. Chem. Soc.*, **62**, 2113 (1940)
[204] Brigl, Mühlschlegel, and Schinle, *Ber.*, **64**, 2921 (1931).

Haworth and co-workers [205] (1938) applied the Weerman degradation (p. 1541) to the crystalline 2,3,5,6-tetramethylgluconamide obtained from the crystalline tetramethyl-γ-gluconolactone. A crystalline cyclic urethane was produced which yielded 2,4,5-trimethyl-*d*-arabinose as a distilled syrup on treatment with cold, dilute alkali. This substance gave a Schiff aldehyde test, and the authors were inclined to regard the compound as an *aldehydo*-structure rather than as a derivative of the propylene oxide ring.

$$
\begin{array}{ccccc}
\text{CONH}_2 & \left[\begin{array}{c}\text{N}{=}\text{C}{=}\text{O}\end{array}\right. & \text{CO} & & \\
| & | & | & & \\
\text{HCOMe} & \text{HCOMe} & \text{NH} & & \text{HC}{=}\text{O} \\
| & | & | & & | \\
\text{MeOCH} \xrightarrow{\text{NaOCl}} & \text{MeOCH} & \text{HCOMe} & \xrightarrow{\text{NaOH}} & \text{MeOCH} \\
| & | & | & & | \\
\text{HCOH} & \text{HCOH} & \text{MeOCH} & & \text{HCOH} \\
| & | & | & & | \\
\text{HCOMe} & \text{HCOMe} & \text{HCO}\text{---}\!\!\rfloor & & \text{HCOMe} \\
| & \left. | \right] & | & & | \\
\text{CH}_2\text{OMe} & \text{CH}_2\text{OMe} & \text{HCOMe} & & \text{CH}_2\text{OMe} \\
& & | & & \\
& & \text{CH}_2\text{OMe} & &
\end{array}
$$
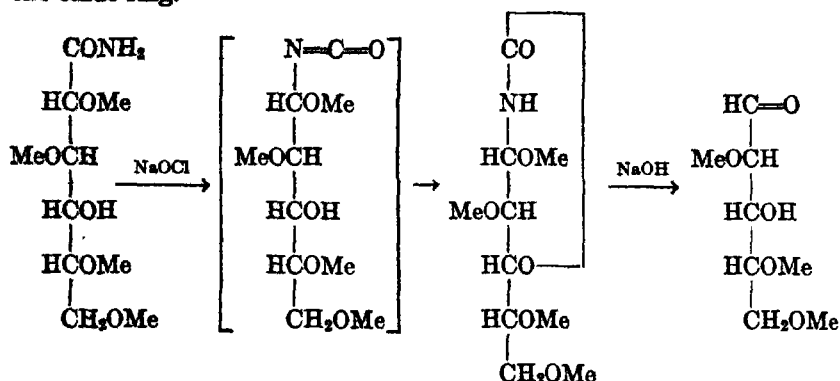
Micheel and co-workers [206] (1933) obtained a galactose tetraacetate with the sixth position open, and this on further acetylation yielded two new pentaacetates of galactose. The pentaacetate was transformed to a new methylgalactoside (β-methyl-*d*-galactoheptanoside) through the acetochloro compound, and this was proved to have a (1,6-) ring by methylation and oxidation. The methylheptanoside had the same low

TABLE V

ISOMERIC PENTAACETATES OF *d*-GALACTOSE

Pentaacetate of:	M.P.	$[\alpha]_D$ Chloroform	2A
α-*d*-Galactopyranose..................	96°	+107°	
β-*d*-Galactopyranose...................	142	+23	+32,700
α-*d*-Galactofuranose (?)...............	87	+61	
β-*d*-Galactofuranose (?)...............	98	−42	+40,200
α-*d*-Galactoheptanose.................	128	−11	
β-*d*-Galactoheptanose.................	112	−103	+35,900
aldehydo-*d*-Galactose.................	121	−25	

[205] Haworth, Peat, and Whetstone, *J. Chem. Soc.*, 1975 (1938).
[206] Micheel and Suckfüll, *Ann.*, **502**, 85 (1933); **507**, 138 (1933); *Ber.*, **66**, 1957 (1933); Micheel and Spruck, *Ber.*, **67**, 1665 (1934).

stability toward acid hydrolysis as the methylfuranosides. Purves and Hudson [207] (1937) have demonstrated that fructopyranosides likewise are hydrolyzed by acids with about the same speed as fructofuranosides. The reactions employed by Micheel are diagrammed below.

```
HC(SEt)₂      HC(SEt)₂           HC(SEt)₂              HC=O
  |             |                  |                    |
HCOH          HCOH               HCOAc                HCOAc
  |             |                  |                    |
HOCH    →     HOCH        →     AcOCH       →         AcOCH      →
  |             |                  |                    |
HOCH          HOCH               AcOCH                AcOCH
  |             |                  |                    |
HCOH          HCOH               HCOAc                HCOAc
  |             |                  |                    |
CH₂OH         CH₂OC(C₆H₅)₃       CH₂OC(C₆H₅)₃         CH₂OC(C₆H₅)₃
```

α- and β-
```
    OH                             Cl    H    · MeO    H
HC<                  HCOAc           \\ C /          \\ C /
    OH                |               |              |
  |                 HCOAc           HCOAc          HCOAc
HCOAc                 |               |              |
  |                 AcOCH           AcOCH          AcOCH
AcOCH       →         |        →      |       →      |        →
  |                 AcOCH           AcOCH          AcOCH
AcOCH                 |               |              |
  |                 HCOAc           HCOAc          HCOAc
HCOAc                 |               |              |
  |                 CH₂O—           CH₂O—          CH₂O—
CH₂OH
```

```
MeO   H          MeO   H
  \\ C /            \\ C /              CO₂H            CO₂H
   |                |                   |               |
 HCOH             HCOMe               HCOMe           HCOH
   |                |                   |               |
 HOCH      →      MeOCH       →       MeOCH    ←       HOCH
   |                |                   |               |
 HOCH             MeOCH               MeOCH           HOCH
   |                |                   |               |
 HCOH             HCOMe               HCOMe           HCOH
   |                |                   |               |
 CH₂O—            CH₂O—               CO₂H            CO₂H
```

The present rather considerable knowledge of the trioses glyceraldehyde and dihydroxyacetone and of the one diose, glycolaldehyde, is due in large part to the careful work of H. O. L. Fischer and co-workers in this very difficult field. The crystalline forms of glycolaldehyde and of glyceraldehyde are dimeric, but molecular-weight determinations show

[207] Purves and Hudson, *J. Am. Chem. Soc.*, **59**, 1170 (1937).

that they pass spontaneously into the monomer in aqueous solution. The derivatives [208] of these aldoses are likewise dimeric. It is probable that the dimeric structures are produced by the carbonyl of one molecule forming a lactol with the hydroxyl hydrogen of a second molecule to produce a six-membered ring of the dioxane type.[209] The depolymerization of dimeric dihydroxyacetone has been studied kinetically by a dilatometric method [210] (1937). The reaction is catalyzed by acids and bases, and its general behavior is similar to the mutarotation of glucose.

$$
\begin{array}{cc}
& \text{CH}_2\text{OH} \\
& | \\
\text{HOCH}\!-\!-\!\text{CH} & \text{CHO} \\
| \quad\quad | & | \\
\text{O} \quad\quad \text{O} \quad\rightleftharpoons\ 2\ & \text{CHOH} \\
| \quad\quad | & | \\
\text{CH}\!-\!\text{HCOH} & \text{CH}_2\text{OH} \\
| \\
\text{CH}_2\text{OH}
\end{array}
$$

Glyceraldehyde

$$
\begin{array}{cc}
\text{HOCH}\!-\!-\!\text{CH}_2 & \\
| \quad\quad | & \text{CHO} \\
\text{O} \quad\quad \text{O} \quad\rightleftharpoons\ 2 & | \\
| \quad\quad | & \text{CH}_2\text{OH} \\
\text{CH}_2\!-\!-\!\text{CHOH} &
\end{array}
$$

Glycolaldehyde
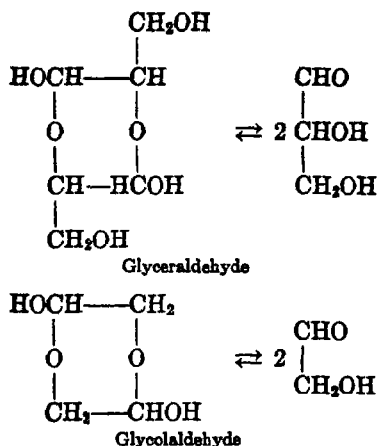
Enolic Structure. One other phase of the glucose structure requires attention, and that is the enolic form. As the general chemistry of the carbonyl group has developed, it has become evident that one of its main reactions is that of enolization.

$$R\!-\!CH_2\!-\!CHO \rightleftharpoons R\!-\!CH\!\!=\!\!CHOH$$
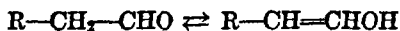
The extent of such spontaneous enolization varies considerably with the nature of the carbonyl compound but is greatly enhanced by alkalinity. There is also good evidence that the enolic form is the intermediate in various reactions, the aldehyde or ketone shifting over to this form as the enol is consumed in the reaction. It would be strange indeed if the aldehyde glucose were an exception to these well-established principles. The distinctive peculiarity of the sugar enolic form is that this is not the usual enol, but an enediol, $-C(OH)\!\!=\!\!C(OH)-$. The indirect evidence for the existence of the enolic sugar structure is impressive and is to be found especially in the complicated reactions that take place when

[208] Fischer, Taube, and Baer, *Ber.*, **60**, 479 (1927); Fischer and Taube, *Ber.*, **60**, 1704 (1927); Fischer and Baer, *Ber.*, **62**, 1749 (1930).

[209] Wohl and Neuberg, *Ber.*, **33**, 3095 (1900); Bergmann and Miekeley, *Ber.*, **62**, 2297 (1929); Fischer and Baer, *Ber.*, **63**, 1744 (1930).

[210] Bell and Baughan, *J. Chem. Soc.*, **1947** (1937).

the reducing sugars are placed in alkaline media, the detailed consideration of which will be reserved for the succeeding chapter (p. 1640). A crystalline derivative of d-glucose-1,2-enediol is the substance obtained by West [211] (1927) on condensing glucose with ethyl acetoacetate. The structure of this compound was established by García González [212] (1934).

$$\text{HOH}_2\text{C}\overset{\overset{\text{H}}{|}}{-}\text{C}\overset{\overset{\text{H}}{|}}{-}\text{C}\overset{\overset{\text{OH}}{|}}{-}\text{C}\overset{\overset{\text{HC}}{\|}}{-}\text{C}\overset{\overset{\text{C}}{\|}}{-}\text{CO}_2\text{C}_2\text{H}_5$$
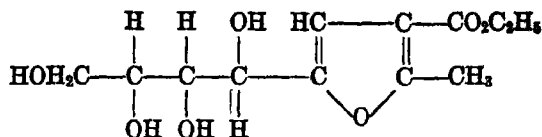
Summary of Ring Structure and Tautomeric Forms. All the preceding evidence shows definitely that glucose is a highly tautomeric substance. To be sure, the two known crystalline forms of this sugar undoubtedly have the pyranose structure, but when these are brought into solution many changes may occur. Evidence has been obtained for all the following glucose structures. The stable or resting stages are the pyranose forms, I and III; IV and VI are perhaps favored by acidity and II and V by alkalinity.

[211] West, *J. Biol. Chem.*, **74**, 561 (1927).
[212] García González, *Anales soc. españ. fís. quím.*, **32**, 815 (1934).

Brigl and Schinle [213] (1933; 1934) have obtained direct evidence for the high degree of tautomerism displayed by fructose. The marked changes in rotation with temperature exhibited by aqueous solutions of fructose indicated that this sugar was highly tautomeric. Brigl and Schinle isolated three crystalline benzoates by the direct benzoylation of fructose and furnished good evidence that they were, respectively:

$$
\begin{array}{ccc}
\text{CH}_2\text{OBz} & \text{CH}_2\text{OBz} & \text{CH}_2\text{OBz} \\
| & | & | \\
\text{HOC} \rule{1em}{0.4pt} & \text{C=O} & \text{HOC} \rule{1em}{0.4pt} \\
| & | & | \\
\text{BzOCH} & \text{BzOCH} & \text{BzOCH} \\
| & | & | \\
\text{HCOBz} & \text{HCOBz} & \text{HCOBz} \\
| & | & | \\
\text{HCOBz} & \text{HCOBz} & \text{HCO} \rule{1em}{0.4pt} \\
| & | & | \\
\text{CH}_2\text{O} \rule{1em}{0.4pt} & \text{CH}_2\text{OBz} & \text{CH}_2\text{OBz} \\
\beta\text{-Fructopyranose} & keto\text{-Fructose} & \text{Fructofuranose} \\
\text{tetrabenzoate} & \text{pentabenzoate} & \text{tetrabenzoate}
\end{array}
$$

KETOSES

d-Fructose is the ketose most widely distributed in nature and is thus the most available of this group of substances. All the known ketoses have the carbonyl group on carbon two. H. O. L. Fischer and Baer [214] (1936) found that an equimolecular mixture of d-glyceraldehyde and dihydroxyacetone underwent an aldol condensation to produce d-fructose and d-sorbose in about equal amounts and in practically quantitative yield. They obtained d-glyceraldehyde [215] by the fission of 1,2,5,6-diacetone-d-mannitol [216] with lead tetraacetate and subsequent acid hydrolysis of the isopropylidene group.

The Lobry de Bruyn dilute alkali interconversion reaction (p. 1641) was used successfully by Montgomery and Hudson [217] (1930) in obtaining the crystalline ketose of lactose (lactulose) and by Austin [218] (1930) for d-glucoheptulose. Another ketose synthesis is the biological method developed by Bertrand. In 1852, the French scientist Pelouze [219] described the isolation of a new ketohexose from the juice of the berries of the mountain ash. This was a readily crystallizable sugar which has

[213] Brigl and Schinle, *Ber.*, **66**, 325 (1933); **67**, 127 (1934).
[214] Fischer and Baer, *Helv. Chim. Acta*, **19**, 519 (1936).
[215] Fischer and Baer, *ibid.*, **17**, 622 (1934).
[216] E. Fischer and Rund, *Ber.*, **49**, 88 (1916); *cf.* v. Vargha, *Ber.*, **66**, 1394 (1933).
[217] Montgomery and Hudson, *J. Am. Chem. Soc.*, **52**, 2101 (1930).
[218] Austin, *ibid.*, **52**, 2106 (1930).
[219] Pelouze, *Ann. chim. phys.*, [3] **35**, 222 (1852).

been named *l*-sorbose. For half a century, attempts to repeat the experiments of Pelouze remained unsuccessful, until Bertrand [220] (1896) found that the mountain ash synthesized merely the alcohol sorbitol and that this was oxidized to the ketose by a bacterium introduced into the fruit by a type of vinegar fly. Bertrand isolated this bacterium, now known as the sorbose bacterium (*Acetobacter xylinum*), and with this in hand it was a rather simple problem for him to prepare the ketose from (dextro)-sorbitol.

This method for ketose synthesis, being biological, is strictly limited in application to those alcohols having a *cis* configuration on carbon atoms two and three or four and five. Bertrand [221] (1904) made crystalline dihydroxyacetone available by the action of the bacterium on glycerol. He also obtained crystalline *l*-glucoheptulose [222] (1928), and a crystalline ketoheptose, perseulose [223] (1909), from perseitol, a naturally occurring heptitol. The known ketose sugars are tabulated in Table VI.

GLYCURONIC ACIDS

The pioneer physiological chemist Schmiedeberg [224] (1879) found that when camphor was fed to an animal it was eliminated in the urine as a bornylglycoside in which the terminal primary hydroxyl group of glucose had been oxidized to the carboxyl group. On hydrolysis, this produced crystalline glucuronic acid lactone,

$$\overset{\displaystyle \lceil \underline{\hspace{1.2cm}} O \underline{\hspace{1.2cm}} \rceil}{CO-CH-CHOH-CH-CHOH-CHOH} \atop \underset{\displaystyle \lfloor \underline{\hspace{2cm}} O \underline{\hspace{2cm}} \rfloor}{}$$
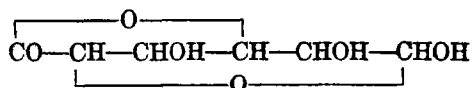
in which the pyranose ring structure is indicated by the methylation experiments of Pryde and Williams [225] (1933) on bornyl glucuronate. According to Quick [226] (1927), glucuronic acid can be most conveniently prepared from bornyl glucuronate, obtained by administering borneol to dogs. Zervas and Sessler [227] (1933) have synthesized glucuronic acid from acetonebenzylideneglucose (I). The free terminal primary alcohol group of I was oxidized with alkaline permanganate solution to the acid II, which on catalytic hydrogenation with palladium produced acetone-

[220] Bertrand, *Bull. soc. chim.*, [3] 15, 627 (1896).
[221] Bertrand, *Ann. chim. phys.*, [8] 3, 230 (1904).
[222] Bertrand and Nitzberg, *Compt. rend.*, 186, 1172 (1928).
[223] Bertrand, *Bull. soc. chim.*, [4] 5, 629 (1909).
[224] Schmiedeberg and Meyer, *Z. physiol. Chem.*, 3, 422 (1879).
[225] Pryde and Williams, *Biochem. J.*, 27, 1197 (1933).
[226] Quick, *J. Biol. Chem.*, 74, 331 (1927).
[227] Zervas and Sessler, *Ber.*, 66, 1326 (1933).

glucuronic acid (III), from which the glucuronic acid lactone (IV) was obtained by mild acid hydrolysis. Fischer and Piloty [228] (1891) had also synthesized IV by the reduction of the lactone of glucosaccharic acid.
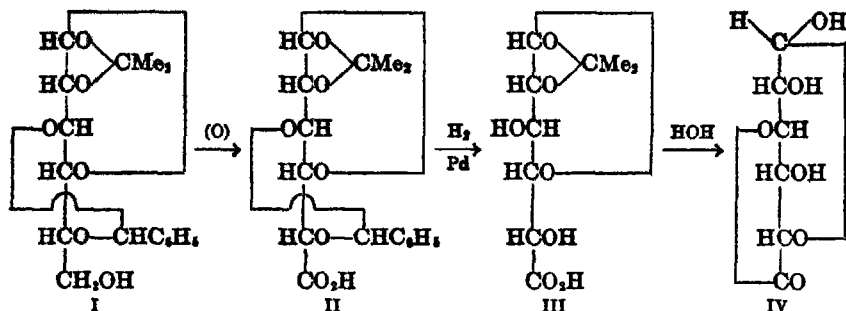
The animal organism has the power of combining substances which are toxic, or which can be oxidized·only slowly, with glucuronic acid and excreting them in the urine. It was once suggested that the substances first form a glucoside with glucose, which then, since the aldehyde group is protected, is oxidized at the other end of the chain. This view can no longer be upheld since Pryde and co-workers [229] (1934) have shown that phenyl- and bornyl-β-glucosides are not converted by the dog to the corresponding glucuronates. The source of glucuronic acid is probably mucin. The place of glucuronic acid in the general scheme of detoxication mechanisms of the body has been studied by Quick [230] (1932).

d-Glucuronic acid has a very widespread occurrence in the plant and animal world; with galacturonic acid, it is a constituent of the plant mucilages and gums. d-Glucuronic acid is found in the type specific polysaccharide of Type III pneumococcus in combination with glucose [231] (Heidelberger and Goebel, 1927); and a similar substance, d-galactopyranose-6-glucuronate, is a partial hydrolysis product of gum arabic. The extended investigations of Levene and co-workers have established the presence of glucuronic acid as a constituent of the carbohydrate portion of the mucoproteins.

d-Galacturonic acid is a component of the fruit pectins, which have been extensively investigated by Ehrlich and his students. Link and co-workers [232] (1931) have described its preparation in crystalline condition from technical citrus pectin. Anderson [233] has made the interesting

[228] Fischer and Piloty, Ber., 24, 524 (1891).
[229] Hemingway, Pryde, and Williams, Biochem. J., 28, 136 (1934).
[230] Quick, J. Biol. Chem., 97, 403 (1932).
[231] Heidelberger and Goebel, ibid., 74, 613 (1927).
[232] Link and Nedden, ibid., 94, 307 (1931); Morell, Baur, and Link, ibid., 105, 15 (1934).
[233] Anderson, ibid., 100, 249 (1933).

observation that both *l*-galactose and *d*-galacturonic acid are hydrolytic products of flaxseed mucilage. Both the *d*- and the *l*-forms of galacturonic acid have been synthesized from diacetone-(1,2;3,4)-galactose by Niemann and Link [234] (1934).
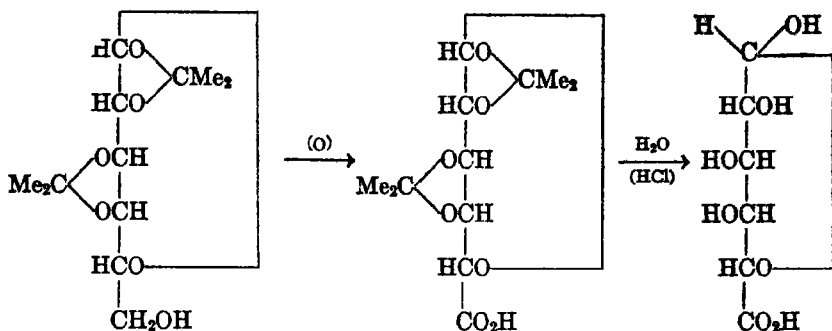
Two crystalline forms of *d*-galacturonic acid are known, and to one of them Ehrlich assigns the aldehyde-hydrate formula. Two crystalline methylgalacturonides have also been prepared (Morell and Link,[235] 1932; Ehrlich and Guttmann,[236] 1933), and evidence based upon kinetics of hydrolysis is offered for a pyranoside ring in the *α*-isomer.

The lactone of *d*-mannuronic acid has been isolated from certain types of seaweed by Nelson and Cretcher [237] (1930). This lactone has also been synthesized by the reduction of *d*-mannosaccharic acid lactone (Niemann and Link,[238] 1933); similar synthetic procedures have yielded *l*-mannuronolactone and *d,l*-alluronic acid (Link and co-workers, 1934 [239]). The ready decarboxylation of uronic acids forms the basis of a widely used analytical method for their estimation, originally devised by Lefèvre and Tollens [240] (1907).

An important result which promises to be useful in the structural determination of uronic acid complexes was the application [241] to methyl galacturonate derivatives of the high-pressure catalytic (copper chromite) procedure [242] for the reduction of esters to primary alcohols. Thus,

[234] Niemann and Link, *ibid.*, **104**, 195, 743 (1934).

[235] Link, *Nature*, **130**, 402 (1932); Morell and Link, *J. Biol. Chem.*, **100**, 385 (1933).

[236] Ehrlich and Guttmann, *Ber.*, **66**, 220 (1933).

[237] Nelson and Cretcher, *J. Am. Chem. Soc.*, **52**, 2130 (1930).

[238] Niemann and Link, *J. Biol. Chem.*, **100**, 407 (1933).

[239] Niemann, McCubbin, and Link, *ibid.*, **104**, 737 (1934); Niemann, Karjala, and Link, *ibid.*, **104**, 189 (1934).

[240] Lefèvre and Tollens, *Ber.*, **40**, 4517 (1907).

[241] Levene, Tipson, and Kreider, *J. Biol. Chem.*, **122**, 199 (1937); Levene and Christman, *ibid.*, **122**, 203 (1937).

[242] Adkins and Connor, *J. Am. Chem. Soc.*, **53**, 1091 (1931); Adkins and Folkers, *ibid.*, **53**, 1095 (1931).

α-methyl-d-galacturonide methyl ester triacetate [243] was converted to α-methyl-d-galactopyranoside.

$$
\begin{array}{ccc}
\text{OMe} & & \text{OMe} \\
| & & | \\
\text{HC}\!\!-\!\!\rule{0.8cm}{0.4pt} & & \text{HC}\!\!-\!\!\rule{0.8cm}{0.4pt} \\
| & & | \\
\text{HCOAc} & & \text{HCOH} \\
| & \xrightarrow{\;\text{H}_2\;} & | \\
\text{AcOCH} & & \text{HOCH} \quad + \text{ MeOH } + \text{ 3EtOH} \\
| & & | \\
\text{AcOCH} & & \text{HOCH} \\
| & & | \\
\text{HCO}\!\!-\!\!\rule{0.8cm}{0.4pt} & & \text{HCO}\!\!-\!\!\rule{0.8cm}{0.4pt} \\
| & & | \\
\text{CO}_2\text{Me} & & \text{CH}_2\text{OH}
\end{array}
$$
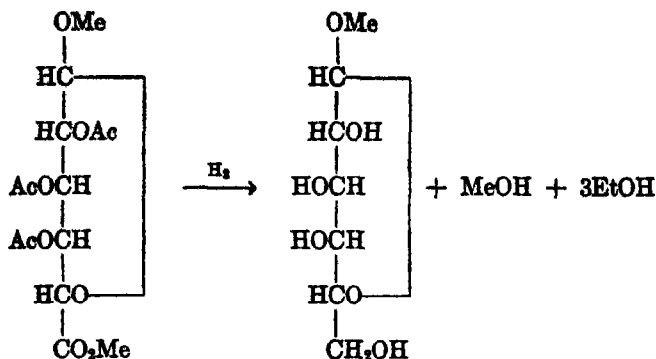
DISACCHARIDE STRUCTURE

Introduction. The term oligosaccharide has been suggested by Freudenberg to denote those polysaccharides which have a definitely known number of component molecular units. The oligosaccharides include the di-, tri-, and tetrasaccharides. Only the more common of the naturally occurring oligosaccharides have had their structures elucidated. Many more undoubtedly exist as constituents of those glycosides that produce several sugars on hydrolysis. A few such rare sugars have been isolated and characterized.[244] A number of synthetic oligosaccharides have been prepared which are not identical with any as yet found in nature. The oligosaccharides are glycosidic condensation products of the monosaccharides, a second molecule of sugar acting as the alcohol, and when hydrolyzed the simple sugars are released and may be identified. Most of the oligosaccharides crystallize as hydrates.

The problems arising in the elucidation of the structure of the disaccharides may be classified under the following heads: (1) identification of the component sugars; (2) which of the component sugars is the alcohol portion; (3) the stereochemical nature (α- or β-) of the glycosidic linkage; (4) which carbon of the alcohol portion is concerned in the glycosidic union; and (5) the ring structure of each of the component sugars. The results of these studies have shown that the common disaccharides fall into three classes as regards the point of glycosidic union: (1) those linked through the reducing groups of each component; (2) those linked to carbon four of the alcohol portion, or the C_4-disac-

[243] Morell and Link, *J. Biol. Chem.*, **106**, 763 (1935).

[244] Zemplén and Gerecs, *Ber.*, **68**, 1318 (1935); Zemplén, *Math. naturw. Anz. ungar. Akad. Wiss.*, **57**, 999 (1938); [*C. A.*, **33**, 4202 (1939)].

charides; (3) those linked to carbon six of the alcohol portion, or the C_6-disaccharides. Furthermore, *d*-glucose is the alcohol portion of each. These structures are tabulated in Table VII.

TABLE VII

STRUCTURE OF THE COMMON DISACCHARIDES

(1) *Disaccharides* linked through the reducing groups

Sucrose — *d*-Glucopyranose — *d*-Fructofuranose

Trehalose — *d*-Glucopyranose — *d*-Glucopyranose
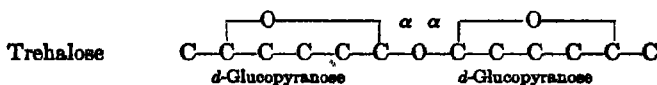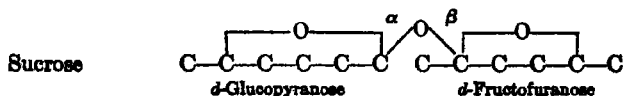
(2) C_4-Disaccharides
Maltose — *d*-Glucopyranose — *d*-Glucopyranose

Cellobiose — *d*-Glucopyranose — *d*-Glucopyranose
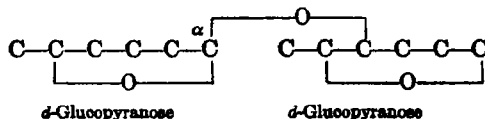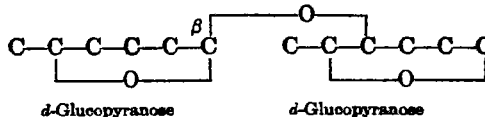
Lactose — *d*-Galactopyranose — *d*-Glucopyranose
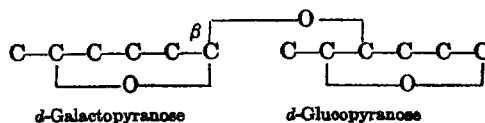
(3) C_6-Disaccharides
Gentiobiose — *d*-Glucopyranose — *d*-Glucopyranose
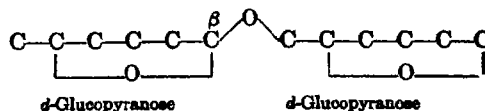
Melibiose — *d*-Galactopyranose — *d*-Glucopyranose
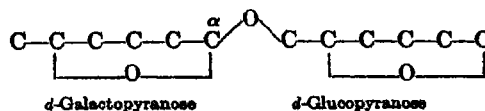
In attacking the problems presented by disaccharide structure, the nature of the component sugars was the problem most readily solved. The stereochemical nature (α- or β-) of the glycosidic linkage was determined by means of enzymic studies and by a consideration of the optical rotations involved; some of these results have not been entirely decisive. The remaining structural problems were solved by methylation methods.

These consisted in methylating all free hydroxyl groups in the molecule, hydrolyzing, and ascertaining the linkage by the nature of the hydrolytic products obtained. This general procedure falls under the head of structural determination by degradative methods. The classical methods of the organic chemist then require that this structure be confirmed by synthesis from intermediates of known structure through controlled reactions. A few selected examples will serve to illustrate these principles.

The methyl sulfate-alkali procedure was successfully adapted to disaccharide methylation by Haworth. Maquenne [245] (1905) had used methyl sulfate for β-glucoside formation, and Haworth adopted the technique of Maquenne for the preliminary formation of a glycoside at low temperatures before using the more stringent conditions required for the remaining hydroxyl groups. A final methylation by the Purdie method was then generally used to ensure complete methylation. More recently, other methods have been applied to the final methylation stages. Thus, the procedure of K. Freudenberg and Hixon [246] (1923), employing the conditions of the Williamson ether synthesis, has been applied [247] (1939) as well as that modification [248] of the Williamson etherification which uses liquid ammonia as a solvent for the formation of the alkoxide. [249]

Methylation Reference Compounds. In determining the nature of the hydrolytic products of the methylated disaccharides, a number of reference substances will be of significance for the examples to be cited.

Tetramethylfructopyranose. n-Tetramethylfructose, a beautifully crystalline sugar, was first prepared by Purdie and Paul [250] (1907) by the methylation of that complex syrupy mixture of fructosides obtained by E. Fischer [251] (1895) through the action of methanol and hydrogen chloride upon fructose. The preparation of crystalline β-methylfructoside by Hudson and Brauns [252] (1916) provided a superior source for the sugar, and its preparation by the methylation and subsequent hydrolysis of this glycoside was reported by Irvine and Patterson [253] (1922). The pyranose or (2,6-) ring structure for this sugar was established by Haworth and Hirst [254] (1926) by the isolation of d-arabotrimethoxyglutaric acid and i-dimethoxysuccinic acid as their crystalline diamides from the nitric acid oxidation of n-tetramethylfructose.

[245] Maquenne, *Bull. soc. chim.*, [3] **33**, 469 (1905).

[246] Freudenberg and Hixon, *Ber.*, **56**, 2119 (1923).

[247] Pacsu and Trister, *J. Am. Chem. Soc.*, **61**, 2442 (1939).

[248] Muskat, *ibid.*, **56**, 693, 2449 (1934).

[249] Irvine and Routledge, *ibid.*, **57**, 1411 (1935).

[250] Purdie and Paul, *J. Chem. Soc.*, **91**, 289 (1907).

[251] Fischer, *Ber.*, **28**, 1145 (1895).

[252] Hudson and Brauns, *J. Am. Chem. Soc.*, **38**, 1216 (1916).

[253] Irvine and Patterson, *J. Chem. Soc.*, **121**, 2146 (1922).

[254] Haworth and Hirst, *ibid.*, 1858 (1926).

```
                                              CO₂H
                                               |
                                             MeOCH
          CH₂OMe                               |
           | /OH                             HCOMe
           C                                   |
           |                                 HCOMe
         MeOCH                                 |
           |              HNO₃                CO₂H
         HCOMe          ────────<
           |                                 CO₂H
         HCOMe                                 |
           |                                 HCOMe
         CH₂O                                  |
                                             HCOMe
                                               |
                                             CO₂H
```
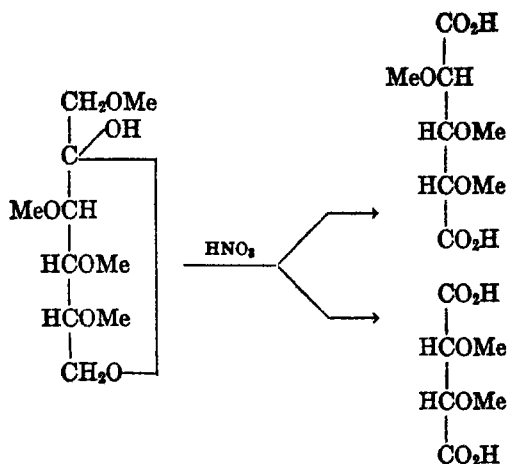
2,3,6-Trimethylglucose. The trimethylglucose (I) having this struc-
ture was prepared in crystalline form by Denham and Woodhouse [255]
(1914) as a hydrolytic product of methylated cellulose (p. 1687). These
workers [256] (1917) considered that the second position·was occupied
because the substance formed no osazone (cf., however, p. 1581), and
that the third carbon was methylated because, on cyanohydrin forma-
tion, demethylation occurred with the formation of a dimethyl lactone
(II). Haworth and Hirst [257] (1921) showed that the sugar was converti-
ble into n-tetramethylglucose (III) on further methylation and hydroly-
sis. A useful derivative of this trimethylglucose was obtained by
Schlubach and Moog [258] (1923), when they isolated its β-methylglycoside
in crystalline form. Further support for the third position carrying a
methyl group was afforded by the isolation of this trimethylglucose from
the hydrolysis products of methylated lactose by Haworth and Leitch [259]
(1918). Since Ruff and Ollendorf [260] (1900) had obtained an osazone
(VII) from the disaccharide (VI) resulting from the degradation of lac-
tose by one carbon atom, then position three must be open in lactose.
Good proof that the sixth position was occupied was provided by Irvine
and Hirst [261] (1922), who obtained a crystalline lead salt of a dimethyl-
saccharic acid (IV) on nitric acid oxidation of this trimethylglucose.

[255] Denham and Woodhouse, ibid., 105, 2357 (1914).
[256] Denham and Woodhouse, ibid., 111, 244 (1917).
[257] Haworth and Hirst, ibid., 119, 193 (1921).
[258] Schlubach and Moog, Ber., 56, 1957 (1923).
[259] Haworth and Leitch, J. Chem. Soc., 113, 197 (1918).
[260] Ruff and Ollendorf, Ber., 33, 1806 (1900).
[261] Irvine and Hirst, J. Chem. Soc., 121, 1213 (1922).

Structure I:

```
 H      OH
  \    /
    C
    |
  HCOMe
    |
  MeOCH
    |
  HCOH
    |
  HCO─┐
    |
  CH₂OMe
    I
```

$HCN + H_2O \longrightarrow$

Structure II:

```
 ┌─CO
 │  |
 │ CHOH
 │  |
 │ HCOMe
 │  |
 └─OCH
    |
  HCOH
    |
  HCOH
    |
  CH₂OMe
     II
```

Structure III:

```
 H      OH
  \    /
    C
    |
  HCOMe
    |
  MeOCH
    |
  HCOMe
    |
  HCO─┐
    |
  CH₂OMe
   III
```

(O)

Structure IV:

```
  CO₂H
    |
  HCOMe
    |
  MeOCH
    |
  HCOH
    |
  HCOH
    |
  CO₂H
    IV
```

$\uparrow$ HOH

Methylated lactose

Structure V:

```
  CHO
    |
  CHOH
    |
  CHOH
    |
  C₉H₁₇O₈
 V. Lactose
```

$\xrightarrow{\ H_2O_2\ }{\ Fe^{+++}\ }$

Structure VI:

```
  CHO
    |
  CHOH
    |
  C₉H₁₇O₈
    VI
```

$\longrightarrow$

Structure VII:

```
  HC═NNHC₆H₅
    |
  C═NNHC₆H₅
    |
  C₉H₁₇O₈
    VII
```
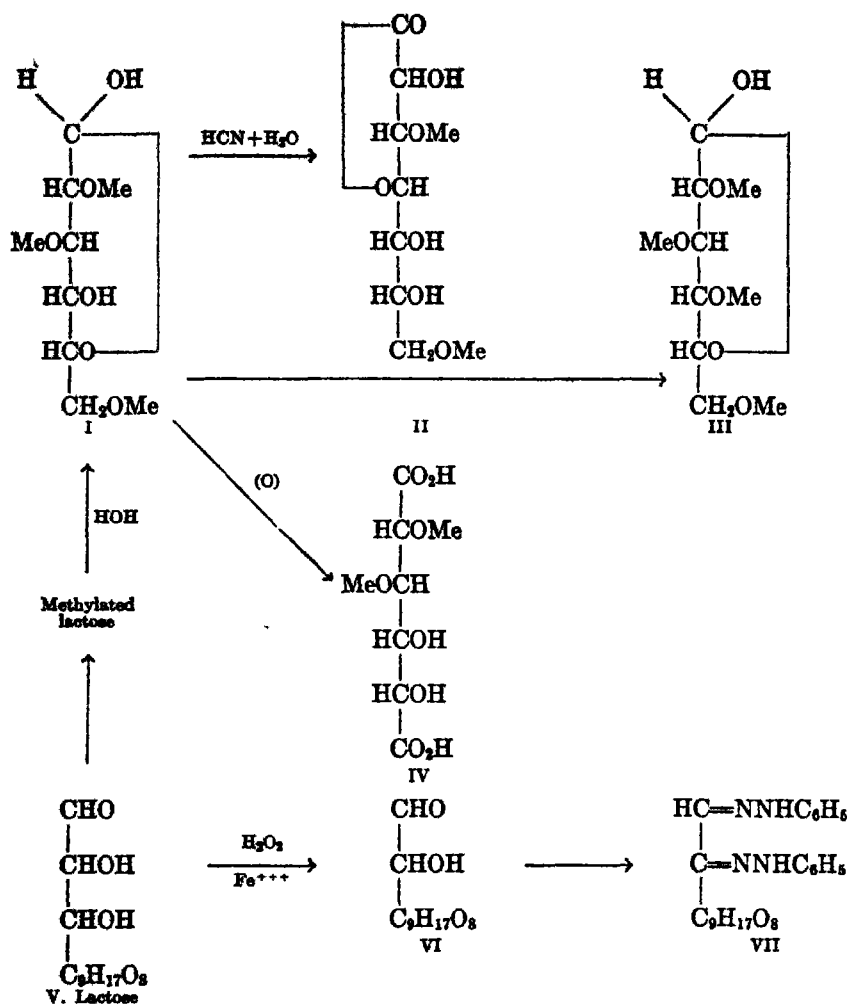
Determination of the Structure of Maltose (4-d-Glucopyranosyl-α-d-glucopyranoside).

Left structure:

```
 H      OH
  \    /
    C
    |
  HCOH
    |
  HOCH
    |
  HCO─┐
    |
  HCO─┘
    |
  CH₂OH
```

Right structure:

```
 H      
  \    /
    C
    |
  HCOH
    |
  HOCH
    |
  HCOH
    |
  HCO─┐
    |
  CH₂OH
```
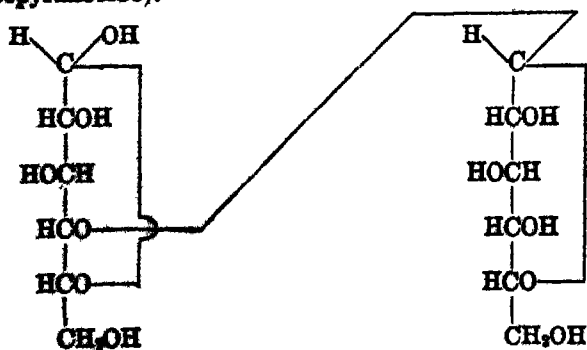
Maltose was isolated by Dubrunfaut [262] (1847) and is produced along with dextrins by the diastatic degradation of starch. It is reducing and produces two moles of d-glucose on hydrolysis. Its hydrolytic enzyme is maltase, and the sugar is therefore considered to possess an α-disaccharide linkage. E. Fischer had classified glycosides on the basis of enzyme specificity, the α-form being the one hydrolyzed by maltase and the β-isomer the one split by emulsin. This useful principle is not always of general application.

As early as 1905, Purdie and Irvine [263] methylated maltose by the silver oxide method. Although oxidation occurred during the methylation process, they succeeded in isolating crystalline n-tetramethylglucose as a hydrolytic product of their methylated substance. Haworth and Leitch [264] (1919) obtained a completely methylated and unoxidized maltose structure by the methyl sulfate procedure, and on hydrolysis of their methylheptamethylmaltoside they verified the results of Purdie and Irvine by obtaining n-tetramethylglucose. The second hydrolytic

2,3,6-Trimethylglucose   n-Tetramethylglucose
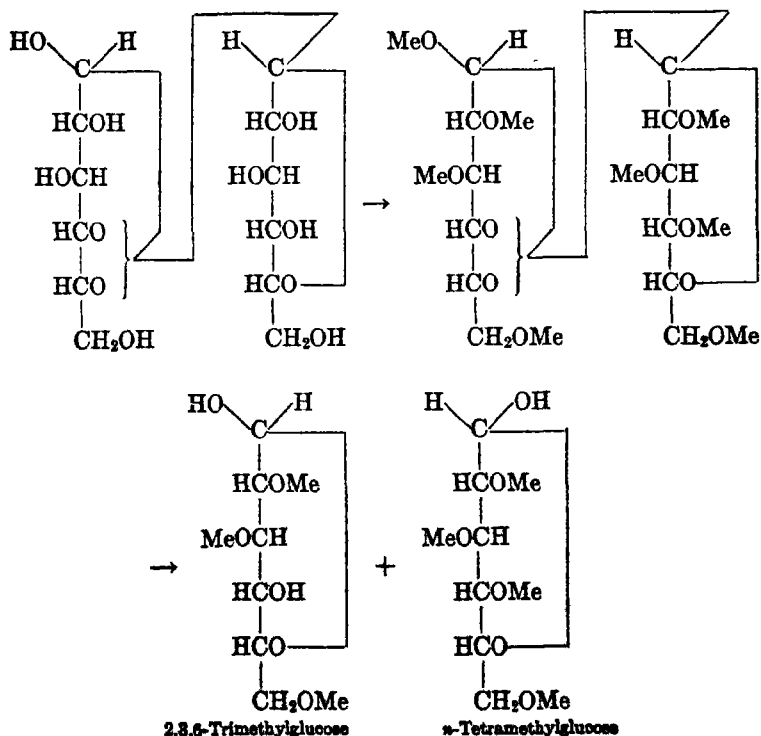
[262] Dubrunfaut, Ann. chim. phys., [3] 21, 178 (1847).
[263] Purdie and Irvine, J. Chem. Soc., 87, 1022 (1905).
[264] Haworth and Leitch, ibid., 115, 809 (1919).

product was isolated in crystalline form and identified as 2,3,6-trimethyl-glucose in 1926 (Irvine and Black;[266] Cooper, Haworth, and Peat[266]).

The isolation of 2,3,6-trimethylglucose as a hydrolytic product of a disaccharide does not definitely complete the structural determination, since the glucose molecule in the reducing portion might exist in either the pyranose or furanose form. It is necessary to prove that one of the two positions, four or five, is involved in the glycosidic linkage and the other is involved in the lactol structure. Definite allocation of the disaccharide linkage was made by Haworth and Peat[267] (1926), who eliminated the troublesome ring in the reducing portion of the maltose molecule through oxidation to the bionic acid. Calcium maltobionate was methylated to form methyl octamethylmaltobionate, and on acid hydrolysis this yielded crystalline n-tetramethylglucose and 2,3,5,6-tetramethylgluconic acid, isolated as its crystalline phenylhydrazide (p. 1560). The structure of the latter follows from its previous formation by the hypobromite oxidation of tetramethylglucofuranose.

Determination of the Structure of Cellobiose (4-d-Glucopyranosyl-β-d-glucopyranoside).
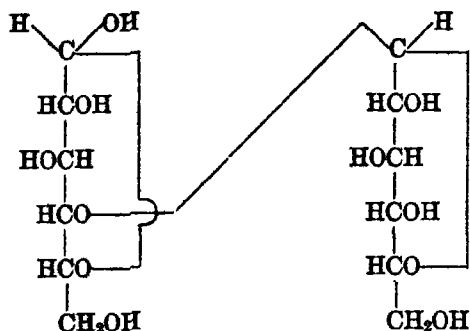
Skraup and König[268] (1901) obtained this sugar in crystalline form by the saponification of its crystalline octaacetate, which had been prepared previously by Franchimont[269] (1879) from the acetolysis of cellulose with acetic anhydride and sulfuric acid. The free sugar is readily obtained crystalline from the saponification of its acetate by sodium ethoxide according to Zemplén[270] (1926). As the best evidence indicates

[266] Irvine and Black, ibid., 862 (1926).

[266] Cooper, Haworth, and Peat, ibid., 876 (1926).

[267] Haworth and Peat, ibid., 3094 (1926).

[268] Skraup and König, Ber., 34, 1115 (1901).

[269] Franchimont, Ber., 12, 1941 (1879).

[270] Zemplén, ibid., 59, 1258 (1926); Zemplén, Gerecs, and Hadácsy, Ber., 69, 1827 (1936).
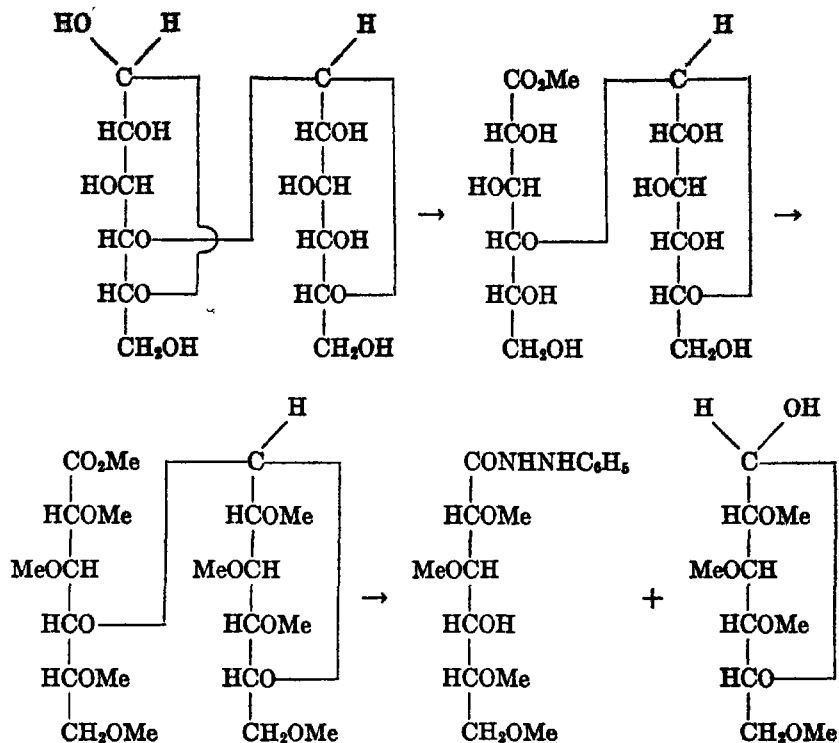
that this sugar is preformed in the cellulose molecule its structure is of fundamental importance. It is reducing and is hydrolyzed by acid or by emulsin into two moles of glucose. Accordingly it has a β-disaccharide configuration.

It differs from maltose only in its glycosidic configuration, and similar methylation procedures were used to prove this point. Crystalline α-cellobiose octaacetate was simultaneously deacetylated and methylated with methyl sulfate and alkali by Haworth and Hirst [371] (1921) to form a crystalline methylheptamethylcellobioside, which on hydrolysis produced crystalline n-tetramethylglucose and the crystalline 2,3,6-trimethylglucose of Denham and Woodhouse.

As with maltose, these results limited the disaccharide linkage to carbon atoms four or five of the glucose molecule. Evidence for the selection of carbon four was given by Haworth, Long, and Plant [372] (1927). Cellobiose was oxidized to calcium cellobionate, and this on complete methylation produced methyl octamethylcellobionate. Hydrolysis of

[371] Haworth and Hirst, J. Chem. Soc., 119, 193 (1921).
[372] Haworth, Long, and Plant, ibid., 2809 (1927).

the latter produced n-tetramethylglucose and 2,3,5,6-tetramethylgluconolactone, which in this case was isolated in crystalline condition (m. p. 26–27°) and also as its crystalline phenylhydrazide.

$$
\begin{array}{c}
\text{CO} \\
| \\
\text{HCOMe} \\
| \\
\text{MeOCH} \\
| \\
\text{HCO} \\
| \\
\text{HCOMe} \\
| \\
\text{CH}_2\text{OMe}
\end{array}
$$

2,3,5,6-Tetramethylgluconolactone
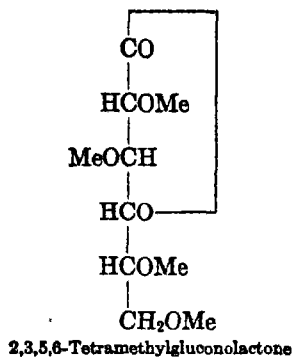
Determination of the Structure of Sucrose (1-α-d-Glucopyranosyl-β-d-fructofuranoside).

$$
\begin{array}{ll}
\text{H} & \\
\quad \diagdown \\
\quad \text{C} & \quad\quad \text{CH}_2\text{OH} \\
\quad | & \quad\quad | \\
\text{HCOH} & \quad\quad \text{OC} \\
\quad | & \quad\quad | \\
\text{HOCH} & \quad\quad \text{HOCH} \\
\quad | & \quad\quad | \\
\text{HCOH} & \quad\quad \text{HCOH} \\
\quad | & \quad\quad | \\
\text{HCO} & \quad\quad \text{HCO} \\
\quad | & \quad\quad | \\
\text{CH}_2\text{OH} & \quad\quad \text{CH}_2\text{OH}
\end{array}
$$
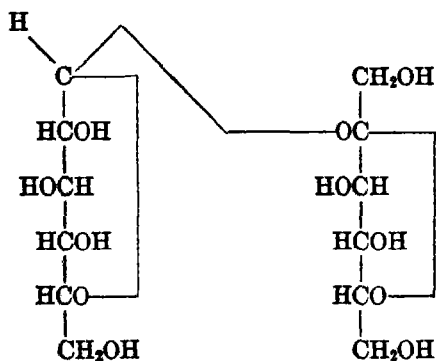
Sucrose or common table sugar has long been known. This sugar is very widely distributed in the plant kingdom and is apparently the transport form of carbohydrate for many plants, as d-glucose is for the animal. The main commercial sources are the sugar cane and the sugar beet, and the final product is sold to the consumer in a very high degree of purity.

Sucrose is a non-reducing disaccharide, and this fact solves immediately the points of attachment of its component sugars as being through their glycosidic carbon atoms. It is hydrolyzed with great ease by dilute acids and also by its enzyme invertase to produce one mole each of glucose and fructose. The stereochemical nature of the two glycosidic linkages has not been determined definitely, but the evidence points to

an α-linkage for the glucose component and to a β-linkage for the fructose portion. From a study of the kinetics of sucrose hydrolysis by concentrated solutions of invertase at low temperatures, Hudson [273] (1909) considered that the glucose component was α-glucose and that the fructose component was a new form of fructose.

Purdie and Irvine [274] (1903) isolated n-tetramethylglucose on hydrolysis of a methylated product obtained by the methylation of sucrose by the Purdie silver oxide method. Haworth [275] (1915) prepared a completely methylated octamethylsucrose, succeeding in this by using successively the methyl sulfate and Purdie alkylation procedures. Sucrose tends to stop methylation at the heptamethyl stage, the eighth methyl group entering with difficulty. Haworth and Law [276] (1916) hydrolyzed this octamethylsucrose under the mildest possible conditions and separated the hydrolytic products by high vacuum distillation. Crystalline n-tetramethylglucose was obtained from the higher-boiling fraction. The lower-boiling fraction proved to be a dextrorotatory tetramethyl hexose which was not identical with the crystalline and highly levorotatory n-tetramethylfructose of Purdie and Paul. Consequently, the fructose component of sucrose must possess a different ring structure from that of ordinary fructose. The ring structure of this so-called γ-fructose was found to be of the furanose (2,5-) type by Avery, Haworth, and Hirst [277] (1927). Haworth [278] (1920) found that the readily obtainable heptamethylsucrose contained a fully methylated fructose portion. Heptamethylsucrose was accordingly used as the best source of γ-tetramethylfructose.

In the work of Avery, Haworth, and Hirst, the syrupy γ-tetramethylfructose (I), obtained by the mild acid hydrolysis of heptamethylsucrose, was oxidized with nitric acid and the oxidation product (II) isolated as the ethyl ester. This substance was a lactol or glucosonic acid which could be methylated to form a non-reducing glycoside, and this in turn produced a crystalline amide. Oxidation of the lactol acid (II) with barium permanganate in acid solution yielded the crystalline (m. p. 29°) trimethyl-γ-d-arabonolactone (III). The enantiomorph of this had been previously obtained by Baker and Haworth [279] (1925) from trimethyl-l-arabinofuranose. Nitric acid oxidation of this lactone yielded (levo)-dimethoxysuccinic acid (IV), characterized as its crystalline amide and

[273] Hudson, J. Am. Chem. Soc., 31, 655 (1909).
[274] Purdie and Irvine, J. Chem. Soc., 83, 1021 (1903).
[275] Haworth, ibid., 107, 12 (1915).
[276] Haworth and Law, ibid., 109, 1314 (1916).
[277] Avery, Haworth, and Hirst, ibid., 2308 (1927).
[278] Haworth, ibid., 117, 199 (1920).
[279] Baker and Haworth, ibid., 127, 365 (1925).

methylamide. This very excellent oxidation work definitely character-
izes the fructose component of sucrose as d-fructofuranose.

```
   CH₂OMe            CO₂H               CO                 CO₂H
    |                 |                  |                  |
  HOC——┐            HOC——┐             CO                MeOCH
    |   │             │   │              │                  │
 MeOCH  │          MeOCH  │           MeOCH              HCOMe
    |   │    →        │   │    →         │        →         │
 HCOMe  │          HCOMe  │           HCOMe              HCOMe
    |   │             │   │              │                  │
  HCO——┘            HCO——┘            HCO——              HCOMe
    |                 |                  |                  |
   CH₂OMe            CH₂OMe            CH₂OMe              CO₂H
    I                 II                III                IV
```
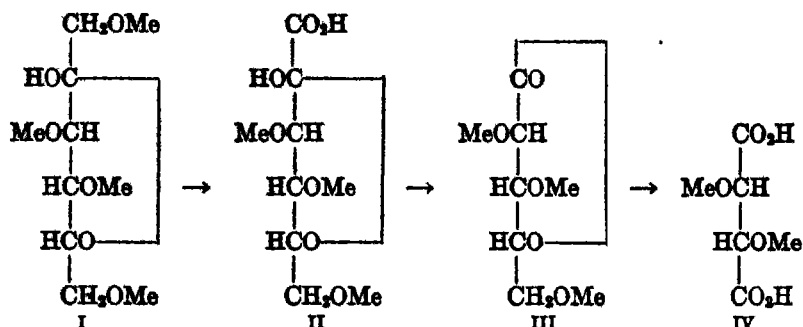
Synthesis of Gentiobiose (6-d-Glucopyranosyl-β-d-glucopyranoside).
Haworth and Wylam [200] (1923) obtained a crystalline methylhepta-
methylgentiobioside by the complete methylation of gentiobiose, and
this on acid hydrolysis produced crystalline n-tetramethylglucose and
2,3,4-trimethylglucose,[201] identified as its crystalline β-methylglycoside.
This indicated that the disaccharide linkage was on carbon six and the
sugar ring on carbon five, or vice versa, with the probability in favor of
the former. Later synthetic experiments decided the carbon six disac-
charide linkage.

The synthetic work of Helferich [202] and his collaborators (1924–
1926) has confirmed the structure of gentiobiose as 6-glucosidoglucose.
Helferich found that triphenylmethyl chloride (called by him trityl
chloride) reacted preferentially with primary alcohol groups. Glucose
reacted with trityl chloride to form a 6-monotrityl ether, the allocation
being proved by treatment with phosphorus pentabromide to form
derivatives of 6-bromoglucose. Fischer and Armstrong [203] (1902) had
previously obtained derivatives of this substance, and Fischer and
Zach [204] (1912) had determined that the bromine was on the terminal
carbon atom by reduction to the methylpentose isorhamnose. Helferich
acetylated 6-tritylglucose and then removed the trityl group by mild
treatment with hydrogen bromide, thus obtaining a glucose structure
with only the sixth position open. Reaction with acetobromoglucose
produced gentiobiose octaacetate, the β-configuration being rendered
probable by the fact that acetobromoglucose produces β-glycosides under

[200] Haworth and Wylam, ibid., 123, 3120 (1923).
[201] Irvine and Oldham, ibid., 127, 2729 (1925); Charlton, Haworth, and Herbert, ibid., 2855 (1931).
[202] Helferich, Z. angew. Chem., 41, 871 (1928); summarising paper.
[203] Fischer and Armstrong, Ber., 35, 833 (1902).
[204] Fischer and Zach, Ber., 45, 3761 (1912).

these conditions. The use of an internal desiccant in this condensation has been found to increase the yield very considerably [228] (Reynolds and Evans, 1938).
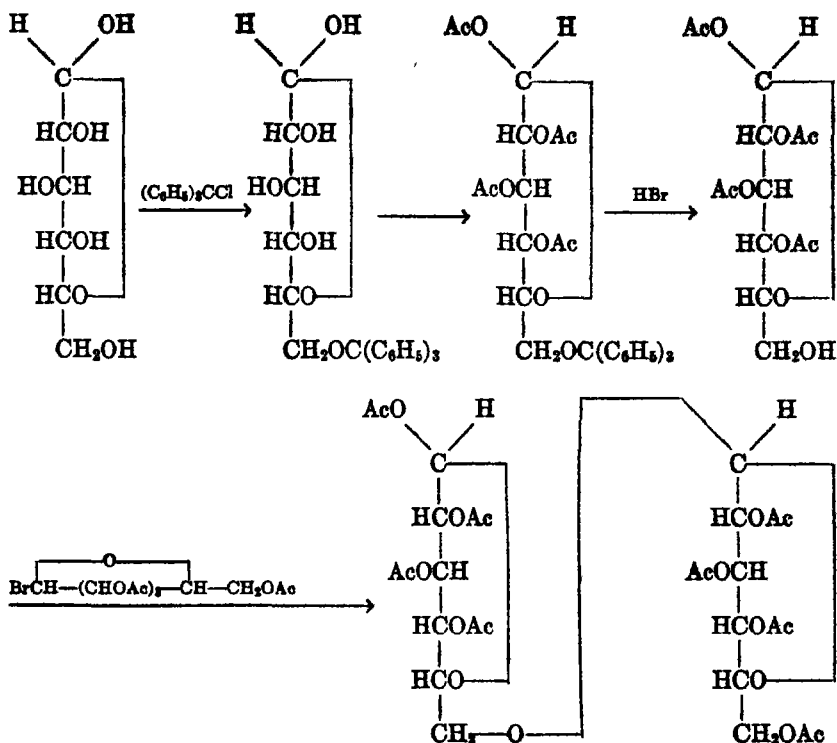
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[228] Reynolds and Evans, J. Am. Chem. Soc., 60, 2559 (1938).

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CHAPTER 21

CARBOHYDRATES II

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CONTENTS

SUBSTITUTED SUGARS

The preceding chapter has been concerned with the structures of the carbohydrates and the methods involved in determining them, and with certain of the reactions of the sugars. The present chapter will devote less attention to questions of structure and configuration, but will discuss certain substituted and derived sugars, and will consider some of their isomerizations and degradations.

In many branches of organic chemistry, the original interest of the subject was either the elucidation of the structure of naturally occurring substances or an inquiry into biological processes. This is particularly true in respect to carbohydrate chemistry, and, although often obscured by the tremendous complexity of the field, the basic interest may be attributed to the extensive distribution in nature of carbohydrates and their derivatives and to their extremely important biological functions. The present discussion will consider the subject from this viewpoint and will devote particular attention to those substances which are of biological as well as of chemical interest.

Esters. A number of carbohydrate derivatives which might be considered of minor chemical importance achieve a significance through their biological associations, and to this class belong many of the esters. Thus while the acetyl and benzoyl esters which have been discussed previously are only infrequently of biological interest, the phosphoric esters on the other hand are of extreme importance and of wide distribution in nature. Some of them have been discovered in muscle metabolism, several have been isolated from fermentation processes, to which they are essential, and a further group are constituents of the nucleic acids. The related glycerol phosphate is a constituent of certain lipides, and the likewise-related phosphoglyceric acid is found in the blood in significant amounts. These last two compounds have further important functions which will be discussed under fermentations.

From studies on fermentation and on muscle enzymes a number of phospho esters have been reported from time to time, but they have not all been authenticated since there is considerable difficulty in securing them in pure state. Four hexose esters for which the structures have been established are the Harden-Young diphosphate (fructose-1,6-diphosphate), the Robison monophosphate (glucose-6-phosphate), the Neuberg ester (fructose-6-phosphate), and the Cori ester (glucose-1-phosphate). Two additional fermentation esters which have been much less investigated are a mannose phosphate and a trehalose phosphate which yields the Robison ester on hydrolysis and must, therefore, be trehalose-6-monophosphate. In the pentose series, ribose-3- and 5-phos-

phates have been secured from yeast nucleic acid degradations, but the 2-desoxyribose phosphate which must be the corresponding constituent of thymus nucleic acid has not as yet been isolated. In very recent years the great activity in the field of fermentation research has led to the isolation of the triose phosphates: dihydroxyacetone phosphate, and glyceraldehyde-2- and 3-phosphates.

Partly in connection with structural studies on the above esters, but mostly because of researches on the possible functions of the phosphoric esters in nature, a number of synthetic phosphates have been prepared. The procedure usually consists in the treatment of a partially substituted carbohydrate with phosphorus oxychloride in the presence of dry quinoline or pyridine or some aqueous alkali. A newer [1] reagent is diphenylphosphoryl chloride; the phenyl groups in the resulting product are removed by catalytic reduction. The directing substituents are removed; the product is isolated and may be purified by precipitation of its barium or calcium salt or by crystallization of its alkaloidal salts. As a typical example [2] there may be illustrated the synthesis of glucose-3-phosphate through the intermediary diacetoneglucose (p. 1557):
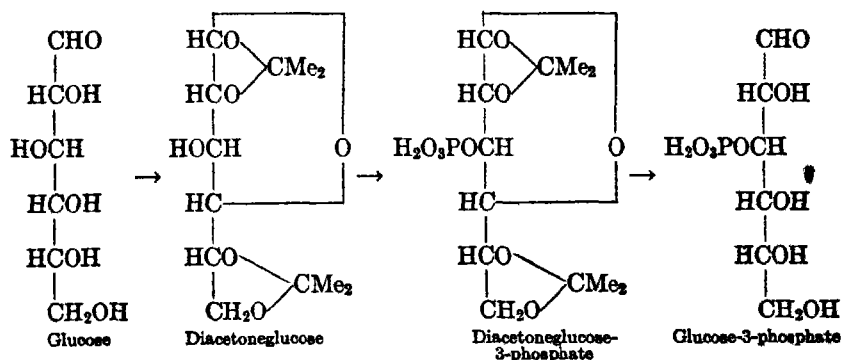
The method is general and requires only that the appropriate partially substituted derivative be available, and that removal of the directing substituents be possible under conditions which leave the phospho group intact. These requirements have been met in a number of instances, and in this manner there have been synthesized the Robison ester mentioned above, as well as glucose-1-, 3-, 4-, and 5-phosphates, leaving only the 2-phosphate unknown in the glucose series. Fructose-1-

[1] Brigl and Müller, *Ber.*, **72**, 2121 (1939).
[2] Levene and Meyer, *J. Biol. Chem.*, **53**, 431 (1922); Komatsu and Nodzu, *Mem. Coll Sci. Kyoto Imp. Univ.*, **7**, Series A, 377 (1924); Nodzu, *J. Biochem.* (Japan), **6**, 31 (1926) Raymond and Levene, *J. Biol. Chem.*, **83**, 619 (1929).

and 3-phosphates, galactose-1- and 6-phosphates, mannose-1-phosphate, d-ribose-5-phosphate (and incidentally uridine- and inosine-5-phosphates), d-xylose-5-phosphate, d-arabinose-5-phosphate, and all three of the triose phosphates previously mentioned have been likewise synthesized. The synthesis, by less clear-cut procedures, of the Harden-Young diphosphate and the Neuberg monophosphate has been reported. Attempts to synthesize d-xylose-3-phosphate resulted instead in the 5-phosphate, migration of the phospho group having apparently occurred during removal of the directing groups. This is the only recorded instance of non-enzymic migration of a phospho group, although migration of other substituents is not uncommon.

In the above syntheses it has been assumed that the phosphorylation was not attended by Walden inversion, and in this connection it is desirable to refer to Robinson's suggestion [3] as to the origin of certain sugars in nature. In experiments on hydrolysis of sugar esters, particularly the tosyl [*] esters, it has been observed that frequently, although not invariably, there occurs a Walden inversion (p. 264), resulting in the formation of a new sugar. Thus, considering only the carbon atom in question:

$$\underset{|}{\overset{|}{HCOH}} \rightarrow \underset{|}{\overset{|}{HCOR}} \xrightarrow[\text{Walden inversion}]{\text{Hydrolysis with}} \underset{|}{\overset{|}{HOCH}}$$
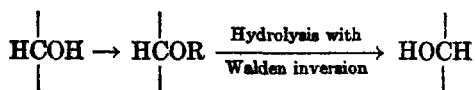
Robinson has suggested that in nature certain sugar esters such as the phosphates are formed, and are then similarly hydrolyzed with attendant Walden inversion, thus producing a new sugar. In this way glucose could yield glucose-4-phosphate and then on hydrolysis could give rise to galactose, thus accounting for the origin of this sugar. Against this ingenious explanation it should be observed that, in all investigations thus far, enzymic dephosphorylation has not produced any sugar other than that originally phosphorylated. Although this does not exclude the possibility that some system actually exists in nature where this change takes place, the reaction has not yet been demonstrated.

A possible explanation of the non-occurrence of Walden inversion during hydrolysis of phospho esters is found in the work of Herbert and Blumenthal.[4] By studies involving the use of water made from the heavy oxygen isotope, they were able to show that cleavage of phosphates occurs between the RO— and the phosphate group: RO + Phospho.

[3] Robinson, *Nature*, **120**, 44 (1927).

[*] "Tosyl" will be used throughout this chapter as an abbreviation for the p-toluene-sulfonyl group, and TlS will be used in formulas.

[4] Herbert and Blumenthal, *Nature*, **144**, 248 (1939).

In this case no Walden inversion would be expected. (See p. 264 *et seq.*)

A second group of esters, owing their particular interest to their biological origin, are the sulfuric esters [5] which occur as constituents of the mucoproteins and in certain seaweeds. In the mucoproteins the non-protein portion has been found to be a complex of glucuronic acid, acetic acid, sulfuric acid, and an aminohexose (chondrosamine or chitosamine). In these compounds the sulfuric acid is apparently attached to a hydroxyl of the hexosamine, but since this sulfuric linkage is most easily severed, the degradation products are all sulfate-free so the point of attachment has not been definitely established. A sulfuric ester of increasing biological interest is heparin, which delays or prevents coagulation of blood. It appears to be a polysulfuric ester of mucoitin.[6] A few synthetic sulfate esters have been prepared by methods analogous to that for the phosphate esters except that the phosphorus oxychloride is replaced by chlorosulfonic acid or sulfuryl chloride. There have been prepared in this manner derivatives of glucose-3-, 6-, and possibly 5-sulfates, and fructose-1- and 3-sulfates. Certain of these esters have been studied by Ohle and co-workers, who consider that the oxidation mechanism of fructose sulfuric (and phosphoric) esters affords insight into the biological processes of carbohydrate degradation.

A further group of naturally occurring esters is that of the tannins. These substances were reported to contain glucose, and, although this was disputed, it seems confirmed by the best available evidence. On the assumption that the small glucose content was due to the presence of several digalloyl residues, Fischer synthesized pentagalloyl- and penta-digalloylglucose and found them to be similar in properties to the natural galloyl tannins.[7] Another synthetic derivative, 1-galloylglucose, has been found to be identical in every respect with a natural product which is associated with the tannins, and a crystalline tannin has been isolated which is apparently a digalloylglucose. In these esters one or more of the carbohydrate hydroxyls are esterified with either gallic, $C_6H_2(OH)_3COOH$, or digallic acid, $HOOCC_6H_2(OH)_2OCOC_6H_2(OH)_3$.

Although not among the naturally occurring esters, the borates are worth mention. From a synthetic standpoint they are of particular interest because in them the substitution is frequently in positions which are different from those of the other common substituting groups. Metaboric acid is employed in the condensations, and two hydroxyls

[5] Levene, "Hexosamines and Mucoproteins," Longmans, Green and Co., London (1925).

[6] Jorpes and Bergström, *J. Biol. Chem.*, **118**, 447 (1937).

[7] Fischer and Freudenberg, *Ber.*, **45**, 915 (1912); Fischer and Bergmann, *Ber.*, **51**, 1760 (1918); *Ber.*, **52**, 829 (1919).

are usually involved

$$\text{CHO} \diagdown \diagup \text{BOH}$$
$$\text{CHO} \diagup$$

. The linkage is easily severed by acid hydrolysis when desired, so that the preparation of new, partially substituted derivatives is facilitated.[8]

A final type of ester which merits consideration is that of the ortho-acetates. These were first discovered[9] in preparing a glycoside from acetobromorhamnose and methyl alcohol in the presence of silver carbonate. They differ from the normal glycosides usually produced by this reaction, being characterized by having one acetyl group resistant to even vigorous alkaline hydrolysis. On the other hand this acetyl and the methyl group are hydrolyzed by the mildest acid treatment, and on this account they were considered to be furanosides, with one acetyl group unaccountably stabilized. The correct explanation was indicated by Braun[10] from a study of ultra-violet absorption bands; the subject was then further studied by Freudenberg and, at about the same time, by Haworth. It was thus shown that one acetyl group is in the ortho-acetate form, being attached to two of the sugar hydroxyls and to the methyl group.

$$\text{O} \begin{cases} \text{—CHO} \diagdown \diagup \text{OCH}_3 \\ \quad | \qquad\quad \text{C} \\ \quad \text{CHO} \diagup \diagdown \text{CH}_3 \\ \quad | \\ \quad \text{CHOCOCH}_3 \\ \quad | \end{cases}$$
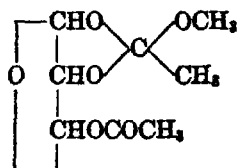
The formation of the orthoacetates is, for some unknown reason, facilitated by having *cis* hydroxyls on carbons two and three of the sugar, so that the orthoacetate is the dominant reaction product with the sugars falling in this category. It has also been observed, however, with sugars not belonging to this type, for example, in the disaccharide, turanose. This sugar is of further interest since Pacsu[11] claims to have secured the two orthoacetates which would be expected, arising from the new asymmetric carbon which has been created in the orthoacetate.

[8] Brigl and Grüner, *Ann.*, **495**, 60 (1932); *Ber.*, **66**, 1977 (1933); *Ber.*, **67**, 1969 (1934); von Vargha, *Ber.*, **66**, 704, 1394 (1933).

[9] Fischer, Bergmann, and Rabe, *Ber.*, **53**, 2385 (1920); Berenshtein and Shapkorskii, *Ukrain. Khem. Zhur.*, **11**, 433 (1936).

[10] Braun, *Naturwissenschaften*, **18**, 393 (1930); *Ber.*, **63**, 1972 (1930); Freudenberg, *Naturwissenschaften*, **18**, 393 (1930); Freudenberg and Schols, *Ber.*, **63**, 1969 (1930); Haworth, Hirst, and Miller, *J. Chem. Soc.*, 2469 (1929); Bott, Haworth, and Hirst, *ibid.*, 1395 (1930).

[11] Pacsu, *J. Am. Chem. Soc.*, **54**, 3649 (1932); see, also, Goebel and Babers, *J. Biol. Chem.*, **110**, 707 (1935).

A further example of these derivatives has been provided by Isbell,[12] who was able to hydrolyze the methyl group in heptaacetyl-4-glucosidomethylmannoside. The resultant compound differed from the ordinary glucosidomannose heptaacetates in that it exhibited no mutarotation, and this was interpreted by Isbell as being due to an orthoacetate structure.

$$
\begin{array}{ccc}
\text{r—CHO} \diagdown & \diagup\text{OCH}_3 & \text{r—CHO} \diagdown \diagup \text{OH} \\
\left| \quad \right| \quad \text{C} & & \rightarrow \quad \left| \quad \right| \quad \text{C} \\
\text{O CHO}\diagup & \diagdown\text{CH}_3 & \text{O CHO}\diagup \diagdown \text{CH}_3 \\
\left| \quad \right| & & \left| \quad \right|
\end{array}
$$
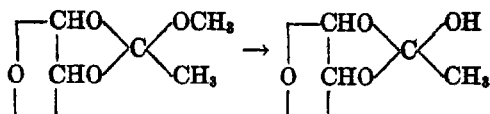
A recent contribution to this interesting field is that of Klingensmith and Evans[13] who condensed acetobromoribose with dihydroxyacetone monoacetate. The product had the orthoacetate structure, yet, in contrast to all previous examples of this group, was unstable to alkali. This was interpreted as being due to rearrangement to the enediol.

In connection with the orthoacetates there may be considered the observed migration of acyl substituents, which has been assumed to pass through the *ortho* form as an intermediate. For example, the migration of the benzoyl group from position three to six in monoacetoneglucose, for which Josephson has provided some excellent data on reaction rates, may be written as follows:

$$
\begin{array}{ccccc}
\left| \quad \right| & & \left| \quad \right| & & \left| \quad \right| \\
\text{O CHOCOC}_6\text{H}_5 & & \text{O CHO}\diagdown & & \text{O CHOH} \\
\left| \quad \right| & & \left| \quad \right| \diagdown & & \left| \quad \right| \\
\text{L—CH} & \rightarrow & \text{L—CH} \quad \diagup\text{C}_6\text{H}_5 & \rightarrow & \text{L—CH} \\
\left| & & \left| \quad \quad \text{C} \diagdown & & \left| \\
\text{CHOH} & & \text{CHOH} \diagup \quad \diagdown\text{OH} & & \text{CHOH} \\
\left| & & \left| \diagup & & \left| \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{O} & & \text{CH}_2\text{OCOC}_6\text{H}_5
\end{array}
$$
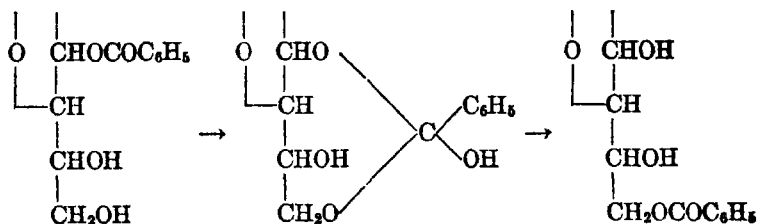
Similar mechanisms have been assumed for other migrations[14] of this type. Acetyl groups, as well as benzoyl groups, have frequently been found to undergo such migrations. On the other hand only one migration of a phospho group in the sugars has been described and there seems to be no authenticated instance in the literature of such migration of alkyl groups. A single example which is of considerable interest, but of a different type, was reported by Ohle,[15] who observed intermolecular migration of a tosyl group: a monotosyl derivative, on treatment with ammonia, was found to give rise to a ditosyl derivative, a part of the material having tosylated the remainder.

[12] Isbell, *J. Research Natl. Bur. Standards*, **7**, 1115 (1931).

[13] Klingensmith and Evans, *J. Am. Chem. Soc.*, **61**, 3012 (1939).

[14] Helferich and Klein, *Ann.*, **455**, 173 (1927); Haworth, Hirst, and Teece, *J. Chem. Soc.*, 1405 (1930); 2858 (1931); Helferich and Müller, *Ber.*, **63**, 2142 (1930); Josephson, *Svensk Kem. Tid.*, **41**, 99 (1929); *Ber.*, **63**, 3089 (1930).

[15] Ohle and Lichtenstein, *Ber.*, **63**, 2905 (1930).

These migrations are of major importance in considerations of structure, and it is necessary to be extremely cautious in basing conclusions upon original positions of the substituents, which may have been altered by the subsequent reactions.

Thio Sugars. Turning from the esters of the sugars to other derivatives occurring in nature, mention may be made of the thio sugars. Apart from the fairly prevalent thioglucosides only one thio sugar from a natural source is known, and this is the thiomethylpentose which occurs in yeast. It is combined as an adenosine nucleoside, but its structure has not yet been established beyond question. Along synthetic lines there have been only two studies on sugars in which the thio group is non-glycosidic, that of Freudenberg on 3-thio- and 3-thiomethylglucose, and that of Raymond on certain sugars in which the thiomethyl group was substituted for the primary hydroxyl.

In the first of these investigations, diacetoneglucose was converted to the xanthogenate, which was methylated, isomerized by heating, hydrolyzed to the thiodiacetoneglucose, and re-methylated to thiomethyldiacetoneglucose. *Acid hydrolysis removed the acetone groups,* giving the free thio- or thiomethylhexose:

$$C_{12}H_{19}O_5OH \xrightarrow[CS_2]{Na} C_{12}H_{19}O_5OCSSNa \xrightarrow{CH_3I} C_{12}H_{19}O_5OCSSCH_3$$

Diacetoneglucose

$$\xrightarrow{Heat} C_{12}H_{19}O_5SCOSCH_3 \xrightarrow{NH_4OH} C_{12}H_{19}O_5SH \xrightarrow{CH_3I} C_{12}H_{19}O_5SCH_3$$

3-Thiomethyl-
diacetoneglucose

$$C_{12}H_{19}O_5SH \xrightarrow{Hydrolysis} C_6H_{11}O_5SH$$

$$C_{12}H_{19}O_5SCH_3 \xrightarrow{Hydrolysis} C_6H_{11}O_5SCH_3$$
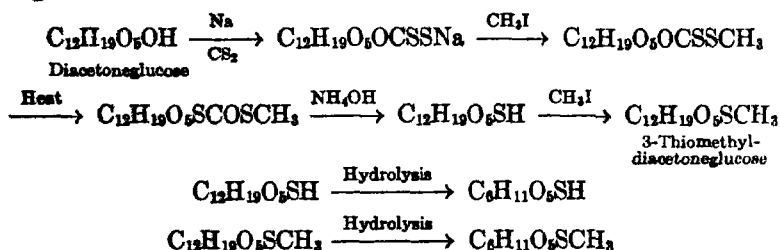
In the second research, 3,5-anhydromonoacetonexylose and 5,6-anhydromonoacetoneglucose were heated with the sodium salt of the appropriate alkyl mercaptan. The anhydro ring was thus opened by addition of the mercaptan, which apparently became attached to the terminal carbon.
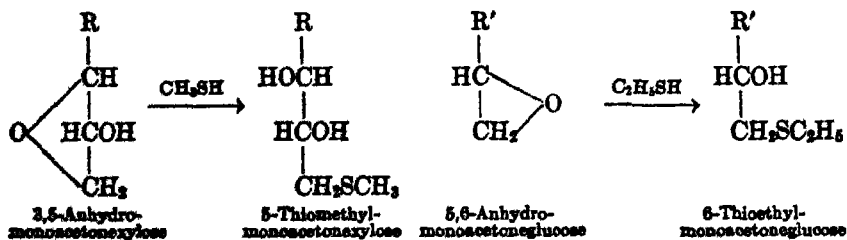
In each of these investigations it was assumed, without experimental confirmation, that Walden inversion had not occurred, and that the thio compound had the configuration of the original sugar.

Amino Sugars. The amino sugars [5] are rather widely distributed in nature, particularly as constituents of muco- and other proteins, and of the polysaccharide, chitin. All the naturally occurring amino sugars are hexoses, and the amino group is invariably on the second carbon atom. Inasmuch as at the present time there is no direct method of establishing the point at which a Walden inversion occurs, the configuration of these compounds cannot be definitely stated. However, on the basis of indirect evidence there seems to be general agreement that chitosamine has the configuration of d-glucose,[16] epichitosamine that of d-mannose, and chondrosamine that of d-galactose. The synthesis of this group of compounds has been effected by adding ammonia and hydrogen cyanide to the appropriate pentose, hydrolyzing the product to the acid, separating the two epimers, converting to the lactone, and reducing to the aminohexose:

$$
\begin{array}{ccccc}
& \text{CN} & \text{COOH} & \text{COOH} & \text{CHO} & \text{CHO} \\
\text{CHO} & | & | & | & | & | \\
| & \text{CHNH}_2 & \text{HCNH}_2 & \text{H}_2\text{NCH} & \text{HCNH}_2 & \text{H}_2\text{NCH} \\
\text{(CHOH)}_3 \rightarrow & | & \rightarrow | & + | & \rightarrow | & + | \\
| & \text{(CHOH)}_3 & \text{(CHOH)}_3 & \text{(CHOH)}_3 & \text{(CHOH)}_3 & \text{(CHOH)}_3 \\
\text{CH}_2\text{OH} & | & | & | & | & | \\
& \text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH}
\end{array}
$$
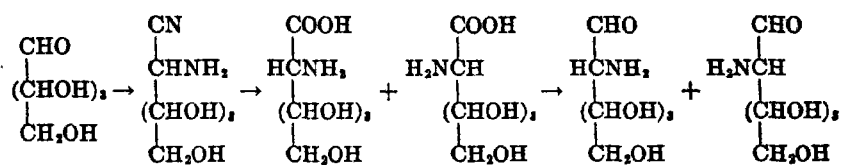
In this way all eight possible 2-amino-d-hexonic acids, but only four of the possible 2-amino-d-hexoses, have been prepared.

A second method of synthesis, more limited in application, is the addition of ammonia to the 2,3-anhydro sugars (see below). In this way 2-aminomethylglucoside [17] and 2-aminomethylaltroside [17, 18] have been obtained. The assumption as to the mechanism of the reaction is clearly stated by Peat and Wiggins [19] and is that opening of the anhydro ring is attended by Walden inversion of only one carbon atom and that this is the carbon to which the entering substituent becomes attached. Thus, in the above cases, the intermediates were derivatives of 2,3-anhydromethylmannoside and 2,3-anhydromethylalloside, respectively:

$$
\begin{array}{cccc}
\text{CHOCH}_3 & \text{CHOCH}_3 & \text{CHOCH}_3 & \text{CHOCH}_3 \\
| & | & | & | \\
\text{CH} \quad O \xrightarrow{\text{NH}_3} & \text{HCNH}_2 \quad O & \text{HC} \quad O \xrightarrow{\text{NH}_3} & \text{NH}_2\text{CH} \quad O \\
O \quad | & | & | \quad O & | \\
\text{CH} & \text{HOCH} & \text{HC} & \text{HCOH} \\
| & | & | & | \\
\text{2,3-Anhydro-} & \text{2-Amino-} & \text{2,3-Anhydro-} & \text{2-Amino-} \\
\text{methyl-} & \text{methylgluco-} & \text{methylallo-} & \text{methyl-} \\
\text{mannoside} & \text{side} & \text{side} & \text{altroside}
\end{array}
$$
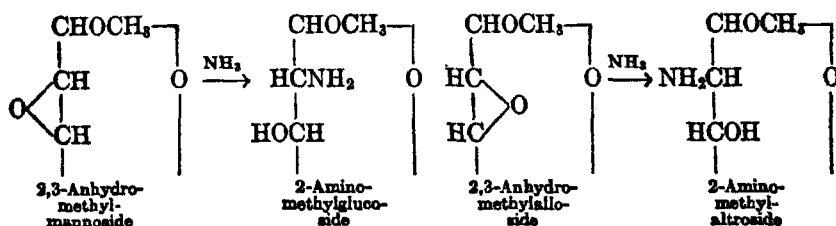
[16] For more direct evidence, see Haworth, Lake, and Peat, *J. Chem. Soc.*, 271 (1939).

[17] Robertson, Meyers, and Tetlow, *Nature*, **142**, 1076 (1938); Robertson and Meyers, *ibid.*, **143**, 640 (1939).

[18] Peat and Wiggins, *J. Chem. Soc.*, 1810 (1938).

[19] Peat and Wiggins, *ibid.*, 1088 (1938).

In many respects the amino sugars resemble the ordinary sugars. Benzylidene derivatives have been prepared as well as certain of the pentaacetates, oximes, semicarbazones, and phenylhydrazones. The acetobromo derivative of chitosamine has been obtained and with phenols gives ordinary pyranosides, hydrolyzable by emulsin. With methyl alcohol, however, the glycoside which is secured is abnormal in that the methyl group is extremely resistant to acid hydrolysis and is not hydrolyzed by emulsin.[20] Further methylation of the glycoside gives a dimethylaminomethylglycoside, and this, on alkaline hydrolysis, loses methyl and amino groups and is converted to glucose. This series of reactions has been interpreted by assuming that the original methylglycoside is really the cyclic compound

$$CH_2OHCHCHCHOHCHOHCHCH$$
$$\overset{\textstyle\ \ \ \ \ }{\underset{CH_3N-O}{\quad}}$$
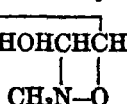
which on methylation and hydrolysis loses the $N(CH_3)_3$ group:

$$CH_2OHCHCHCHOHCHOHCHCH$$
$$(CH_3)_3N-O$$

$$\xrightarrow{H_2O} CH_2OHCHCHOHCHOHCHOHCHOHCHO \text{ (Glucose)}$$
$$+$$
$$(CH_3)_3N$$
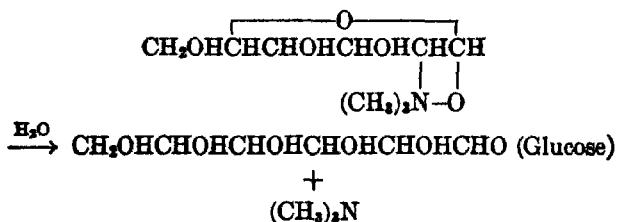
This reaction is of threefold interest: first, because it has been used as a basis for postulating a structure for the polysaccharide chitin; second, because of its possible relationship to the occurrence, in nature, of betaine, CH_2-CO, and similar compounds; and third, because it

$$(CH_3)_3N-\!-\!-O$$
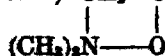
would establish, in the absence of any Walden inversion, the configuration of chitosamine. In this last connection it is interesting to see that precisely the opposite correlation (i.e., to mannose) results from a different series of reactions. Thus, if the same aminomethylglycoside used above is converted into the benzylidene derivative, and this then treated with nitrous acid in the presence of sodium nitrite to avoid excess acidity, the methyl and amino groups are simultaneously eliminated and a benzylidenehexose results. Mild acid hydrolysis removes the benzylidene group and mannose is secured. Since glucose resulted in the former reaction it is evident that in one or the other a Walden inversion has taken place, and in the absence of supplementary data no conclusions as to configuration are possible.

[20] Irvine and Hynd, *ibid.*, **109**, 41 (1913).

Although the only amino sugars found in nature have the amino group in position two, those in which it is in other positions have been prepared synthetically. Thus 1-aminoglucose (glucosimine) has been obtained from glucose and alcoholic ammonia, and subsequently [21] a general synthesis of the 1-aminoaldose derivatives was reported which involves simply dissolving the aldose in liquid ammonia and evaporating the excess solvent. On the basis of the behavior of the oximes and hydrazones of the aldoses, it may be assumed that these compounds can exist both in ring and open-chain forms:

$$\overset{\displaystyle \overline{O}}{CH_2OHCHCHOHCHOHCHOHCHNH_2}$$

or

$$CH_2OHCHOHCHOHCHOHCHOHCH{=}NH$$
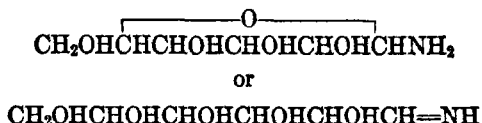
However, this problem has not been deeply explored. Another 1-amino sugar is the 1-aminofructose which was obtained by Fischer by reducing glucosazone with zinc dust and acetic acid:

$$\underset{\text{Glucosazone}}{RC({=}NNHC_6H_5)CH{=}NNHC_6H_5} \rightarrow \underset{\text{1-Aminofructose}}{RCOCH_2NH_2}$$

In addition to these 1-amino sugars, hexosamines with the amino group in position three have been prepared synthetically. The first of these is obtained by treatment of 2-bromo-, 2-chloro-, or 2-tosyl-β-methylglucoside with ammonia.[22] In each case the amino group becomes attached to carbon three, presumably through intermediate formation of an anhydro compound. The product was named "methyl epiglucosamine" by Fischer, although this nomenclature is unfortunate in that it indicates a 2-aminohexose, epimeric with glucosamine. This is quite erroneous as was shown by Levene and Meyer, who prepared the osazone and found that the amino group had been retained.[23]

It is evident that, in the above reaction, there is a possibility of Walden inversion either during formation of the ethylene oxide ring or in its opening by the ammonia, so that epiglucosamine might have any one of four possible configurations. It was deduced by Freudenberg [24] that the most probable one was that of altrose, and later opinions confirm this. On the basis of the mechanism formulated above for the production of the 2-amino sugars from the 2,3-anhydro sugars, here the

[21] Muskat, J. Am. Chem. Soc., 56, 693 (1934).

[22] Fischer, Bergmann, and Schotte, Ber., 53, 516, 539 (1920); Bodycote, Haworth, and Hirst, J. Chem. Soc., 151 (1934).

[23] Levene and Meyer, J. Biol. Chem., 55, 221 (1923).

[24] Freudenberg and Doser, Ber., 58, 294 (1925); Freudenberg, Burkhart, and Braun, Ber., 59, 714 (1926).

intermediate must be 2,3-anhydromethylmannoside (formed from 2-tosylglucose by Walden inversion of carbon-two) which then adds ammonia with Walden inversion of carbon-three:

```
   ┌───CHOCH₃        ┌───CHOCH₃              ┌───CHOCH₃
   │                 │                       │
 O │  HCO·TlS   ──▶ O │   ╱CH     NH₃    O │  HOCH
   │               O⟨          ──▶            │
   │  HOCH           │   ╲CH                  │  HCNH₂
   │                 │                        │
```

| 2-Tosylmethyl-glucoside | 2,3-Anhydro-methylmannoside | 3-Aminomethyl altroside (methyl epiglucosamine) |
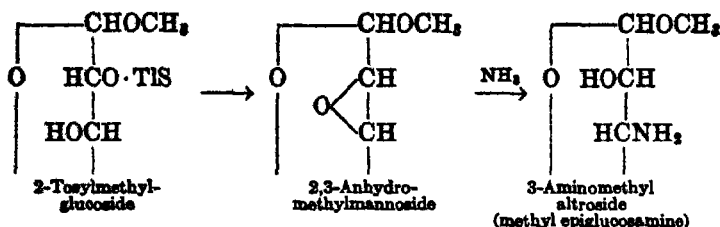
In similar fashion 3-aminomethylglucoside has been obtained by action of ammonia on 2,3-anhydro- and on 3,4-anhydromethylalloside.

```
   ┌───CHOCH₃           ┌───CHOCH₃            ┌───CHOCH₃
   │                    │                     │
 O │  HC╲        NH₃  O │  HCOH    NH₃     O │  HCOH
   │    │ ╲O    ──▶     │          ◀──        │
 O │  HC╱              O│  NH₂CH           O │  HC╲
   │    │               │                     │    │ ╲O
   │  HCOH              │  HCOH                │  HC╱
   │                    │                     │    │
```
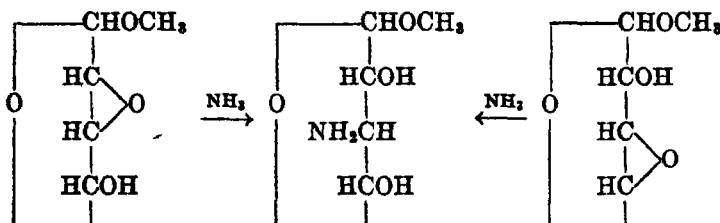
An interesting peculiarity of epiglucosamine is the behavior of its glycoside on attempted acid hydrolysis. Fischer and co-workers considered the glycoside to be non-hydrolyzable, even with rather strong acid, as no reducing sugar was secured. This view was corrected by Levene, who showed that the methyl group was split off in the usual fashion, but that the free sugar underwent spontaneous loss of water, forming a non-reducing anhydro sugar in which the amino group was retained. The structure of this anhydro amino sugar has not been elucidated.

By the action of ammonia on 3-tosyldiacetoneglucose, Freudenberg [24] obtained a 3-aminohexose which was not identical with epiglucosamine. Here the reaction appears to be confined to carbon atom three, with no anhydro formation, and the only uncertainty concerns the occurrence or non-occurrence of Walden inversion. In the former case the product would be 3-aminoallose, and in the latter 3-aminoglucose. Although indirect evidence suggested that the former was the correct configuration, the most recent opinion [18] is that it is more probably 3-aminoglucose.

Two 6-aminohexoses have also been synthetically prepared: those of glucose and of galactose. They result upon treatment of the 6-halogeno- or 6-tosylhexose (usually an acetone derivative or the glycoside) with

ammonia.[25] Since in this series the reaction is confined to a non-asymmetric carbon there is no possibility of Walden inversion and the configuration of these sugars can be stated with certainty.

Brief mention may be made in this connection of lactoflavin, which is one of the components of the vitamin B complex. This interesting substance has been shown to be a derivative of 1-amino-d-ribitol and not only has it been synthesized but so have several isomeric derivatives. In the flavin the amino group of the sugar forms a part of an isoalloxazin nucleus. It has been found that substitution of d- or l-arabinose for d-ribose reduces the biological activity, while the d-xylo derivative is inactive.

Before leaving the amino sugars it is necessary to mention one of their most interesting reactions, that with nitrous acid. In one of the examples cited above, treatment with nitrous acid produced the usual conversion of an amino to a hydroxyl group, and a hexose resulted. This is not generally true, however; more often there is a simultaneous loss of water and anhydro sugars are formed. These products will be more fully discussed in a later section devoted to the anhydro sugars.

DERIVED SUGARS

From the amino sugars attention may be turned to a group of substances which are important for their chemical as well as for their biological associations. They are derived from the monoses by removal of the elements of water, or an oxygen atom, or both water and an oxygen atom. Their relationships may be indicated as follows:

PRODUCT	FORMED FROM MONOSES BY ELIMINATION OF	ETHYLENIC LINKAGE IN PRODUCT
Anhydro sugars	H—OH	None
• Glycoseens	H—OH	One
Glycals	HO—OH	One
Desoxy sugars	O	None

Anhydro Sugars. The anhydro sugars, as indicated above, may be considered as being derived from the monoses by elimination of the elements of water.

$$C_6H_{12}O_6 - H_2O \rightarrow C_6H_{10}O_5$$

However, in the reaction an internal ether is formed, and this new ring might be, a priori, between any two carbon atoms. Actually a great variety of anhydro rings have been reported, covering all the possible forms from ethylene oxide to hexylene oxide.

[25] Fischer and Zach, Ber., 44, 132 (1911); Ohle and v. Vargha, Ber., 61, 1207 (1928).

In the *ethylene oxide* series the 1,2-anhydro derivatives have been given the special name α-glycosans by Pictet,[26] who prepared them by heating the sugars under reduced pressure to eliminate the elements of water. They were reported by these workers as being reasonably stable, and could in fact be crystallized from methyl alcohol. A reaction which was claimed to prove their structure was the addition of methyl iodide to form substances which were apparently 2-iodomethylglycosides.

Derivatives of this ethylene oxide type are presumably the intermediates in the oxidation of glycals (see below) by peracids. When glucal is treated with moist perbenzoic acid the major product is mannose; with dry perbenzoic acid followed by methyl alcohol the product is largely a methylmannoside. The reactions are presumably the following:
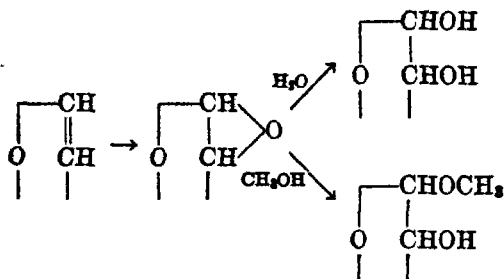
In view of these results, the relative non-reactivity of the α-glycosans described by Pictet is surprising. Brigl,[27] however, has synthesized an acetyl derivative in the following manner:
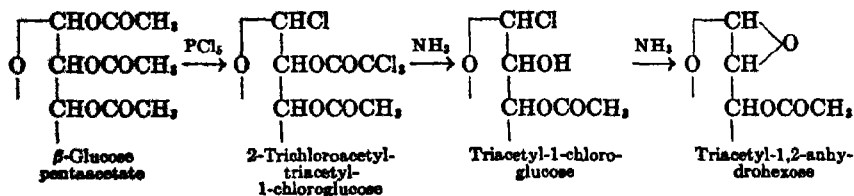
This derivative exhibits the expected reactivity, the anhydro ring being readily opened by various reagents, such as methyl alcohol and acetic anhydride. To explain the difference in reactivity between this substance and Pictet's α-glucosan, it has been assumed that the latter is 1,2-anhydroglucose and that Brigl's compound and the glucal intermediate are 1,2-anhydromannose.

Of the other possible ethylene oxide derivatives, 5,6-anhydromonoacetoneglucose is the best known, being easily prepared from the

[26] Pictet and Castan, *Helv. Chim. Acta*, **3**, 645 (1920) ; *Compt. rend.*, **171**, 243 (1920).

[27] Brigl, *Z. physiol. Chem.*, **116**, 1 (1921).

6-tosyl derivative by careful treatment with sodium methoxide. The ring is a little more difficult to open than that in the 1,2-anhydro compound (of Brigl), but additions may be effected without much trouble. 6-Methylglucose is formed with sodium methoxide, 6-aminoglucose with ammonia, 6-thiomethylglucose with sodium methyl mercaptan (CH_3SNa), and apparently two substances with acetobromoglucose.

These reactions may be indicated as follows:
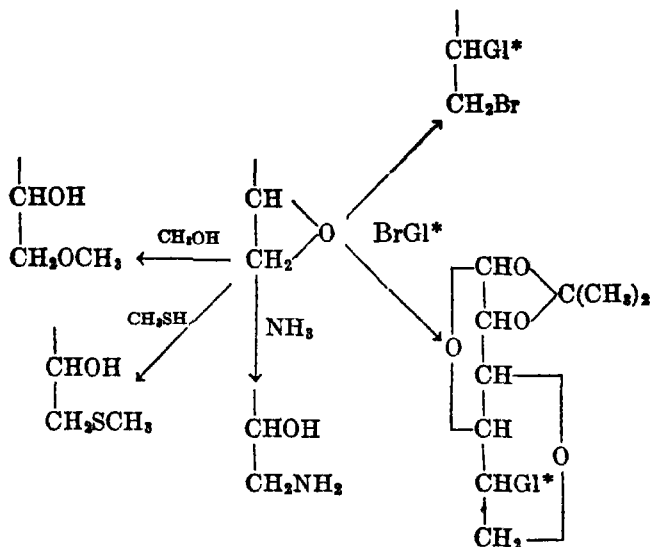
A fact of considerable theoretical importance is that the 5,6-anhydroglucose derivative, on alkaline hydrolysis,[28] gives a mixture of glucose and idose, but on acid hydrolysis only glucose. In this connection may be mentioned the interesting observations of Levene and Walti, who found that acid hydrolysis of optically active propylene oxide gave an excess of one optical isomer, but that on alkaline hydrolysis the opposite form predominated.

A further series of ethylene oxide sugars are the 2,3-anhydro sugars which have already been discussed under the 2- and 3-amino sugars, for which they act as intermediates. Robertson [29] found that appropriate derivatives of 2-tosylglucose could be converted to a 2,3-anhydromannoside and of 3-tosylglucose to the 2,3-anhydroalloside, the reaction in

---

* "Gl" is used here as an abbreviation for the tetraacetylglucose radical, and "BrGl" for acetobromoglucose.

[28] Ohle and von Vargha, *Ber.*, **62**, 2425 (1929).

[29] Robertson and Griffith, *J. Chem. Soc.*, 1193 (1935).

each case being attended by a Walden inversion of the carbon atom to which the tosyl residue was originally attached:

| 2-Tosyl-glucoside | 2,3-Anhydro-mannoside | 3-Tosyl-glucoside | 2,3-Anhydro-alloside |
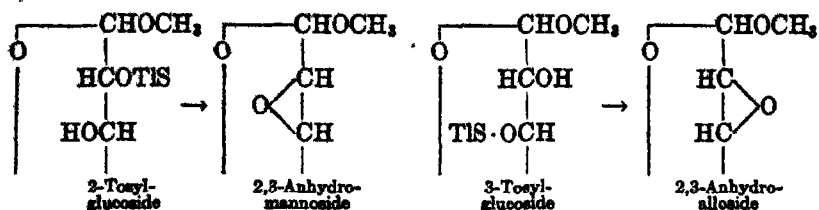
The rule, stated by Ohle and Schultz [30] and substantiated by the work of Müller,[31] is that ethylene oxide rings are formed only if the hydroxyl and sulfonyl groups are *trans*.

Peat and Wiggins,[18] in addition to the above products from the 3-tosylglucoside, also obtained a 3,4-anhydroalloside and a 3,6-anhydro-glucoside. In the former case a Walden inversion has again occurred but in the latter it has not. Ohle and Wilcke,[32] however, interpret the reaction as taking place in two steps, Walden inversion occurring in each step:

| 3-Tosylglucoside | 2,3-Anhydro-alloside | 3,6-Anhydro-glucoside |
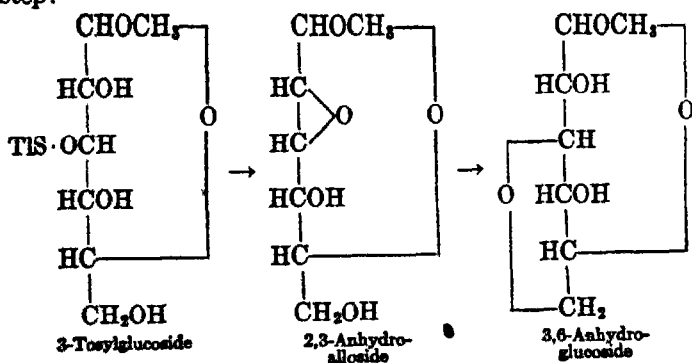
Robertson [29] has also described the formation from galactose of two 2,3-anhydro compounds, but their configurations have not been established.

Analogously to the addition of ammonia, the ethylene oxide sugars react with sodium methoxide to form methyl ethers, with anhydrous hydrogen chloride to give chloro sugars, or with aqueous alkali to introduce hydroxyl groups. For example the conversion of glucose to galactose, suggested by Robinson (p. 1608) as perhaps occurring enzymically through an intermediate ester, has actually been accomplished. Treatment of 2,3-dibenzoyl-4-tosyl-6-triphenylmethylglucose with alkali gives

[30] Ohle and Schultz, *Ber.*, 71, 2302 (1938).
[31] Müller, Mórica, and Verner, *Ber.*, 72, 745 (1939).
[32] Ohle and Wilcke, *Ber.*, 71, 2316 (1938).

a 3,4-anhydro derivative, and this on acid hydrolysis gives a mixture of d-glucose and d-galactose.[32] In similar fashion monoacetone-3-tosyl-fructopyranose can be converted to a 3,4-anhydro derivative which with sodium methoxide gives d-sorbose, or with sodium hydroxide, a mixture of d-sorbose and d-fructose.

Of the *propylene oxide* anhydro sugars only two have been described. One of these is 2,4-anhydroglucose, formed by a complex reaction on treatment of 1,6-anhydroglucose with concentrated hydrochloric acid, and the other is monoacetone-3,5-anhydroxylose, formed from mono-acetone-5-tosylxylose with sodium methoxide.

| 1,6-Anhydroglucose | 2,4-Anhydroglucose | Monoacetone-5-tosylxylose | Monoacetone-3,5-anhydroxylose |
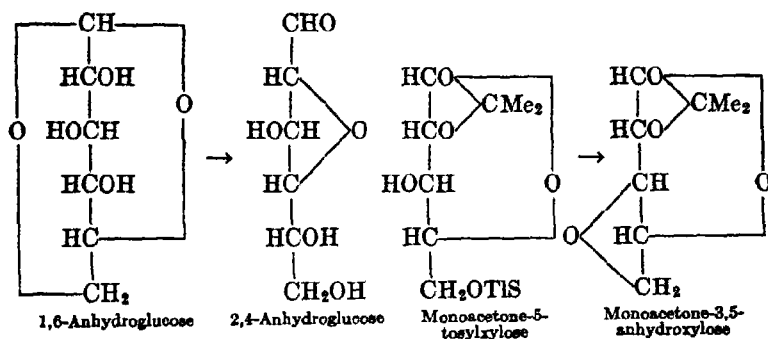
In each of these the ring is relatively unstable; the first can be hydro-lyzed with dilute acid to glucose, while the second adds sodium me-thoxide or sodium methyl mercaptan to form 5-methyl- and 5-thiomethyl-monoacetonexylose, respectively.

The most stable anhydro rings are the *butylene oxide* type, the 2,5- and the 3,6-anhydro sugars in the hexose series. As previously men-tioned, these result on treatment with nitrous acid of the 2- and 3-amino-hexoses, elimination of water being spontaneous. It has been proved by Levene that the substance thus formed from epichitosamine is 2,5-an-hydroglucose whereas that from chitosamine is 2,5-anhydromannose. This proof consists in an experimental determination of configuration, entirely analogous to that used for the monoses themselves, and involves no considerations of Walden inversion.

In addition to this method of formation, the 3,6-anhydro compounds have also been secured by removing the elements of hydrogen bromide from the methyl-6-bromoglycosides by treatment with alkali. In the case of glucose the product thus prepared is not identical with that ob-tained by deaminizing epiglucosamine, but appears rather to be epi-meric.

[32] Oldham and Robertson, *J. Chem. Soc.*, 685 (1935).

One of the 3,6-anhydroglucoses has been hydrolyzed by dilute acids, glucose being regenerated, but the 2,5-anhydro rings are so stable that their hydrolysis has not yet been accomplished.

A substance which may be considered as being both a *butylene* and an *amylene* oxide is the extremely interesting compound prepared by Hess and Neumann.[14] They treated 2,3,6-trimethyl-4-tosylglucose with alkali, the tosyl group was eliminated, and a 1,4-anhydro compound resulted. Since the original 1,5-ring is retained, the product is simultaneously a furanose and a pyranose. Interestingly, on treatment with hydrobromic acid, the rings are opened, the methyl groups are split off, and there is a simultaneous Walden inversion on the fifth carbon, giving rise to *l*-idose.
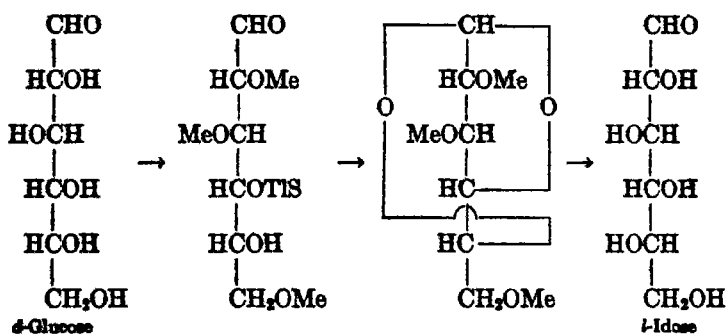
The best known of the 1,6-anhydrohexoses is the levoglucosan[15] of Pictet, prepared by destructive distillation of starch or other polysaccharides under reduced pressure. The compounds belonging to this series may also be prepared synthetically[16] by adding trimethylamine to the acetobromo derivative and then hydrolyzing with alkali, whereby the acetyl groups and the trimethylammonium group are split off and the 1,6-anhydro derivative is secured.
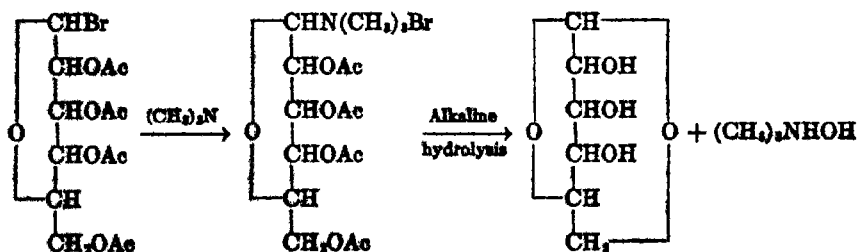
[14] Hess and Neumann, *Ber.*, **68**, 1360 (1935); **72**, 137 (1939).

[15] Pictet and Sarasin, *Helv. Chim. Acta.*, 1, 87 (1918); Pictet and Cramer, *ibid.*, **3**, 640 (1920). See, also, Tanret, *Bull. soc. chim.*, [3] 11, 949 (1894) and Vongerichten and Müller, *Ber.*, **39**, 241 (1906).

[16] Micheel, *Ber.*, **62**, 687 (1929); Karrer and Smirnoff, *Helv. Chim. Acta.*, 4, 817 (1921).

Opening of the 1,6-ring may be effected by various reagents, for example by acetyl bromide, producing triacetyl-1,6-dibromoglucose, which in turn may be converted to the glycoside for use in various syntheses.

$$
\begin{array}{ccc}
\text{1,6-Anhydroglucose} & \text{Triacetyl-1,6-} & \text{Triacetyl-6-bromo-} \\
& \text{dibromoglucose} & \beta\text{-methylglycoside}
\end{array}
$$

Reaction scheme:

Left structure (1,6-Anhydroglucose): CH—, CHOH, CHOH, CHOH, CH—, CH₂ (with O bridges)

$\xrightarrow{CH_3COBr}$

Middle structure (Triacetyl-1,6-dibromoglucose): CHBr—, CHOAc, CHOAc, CHOAc, CH—, CH₂Br

$\xrightarrow[AgCO_3]{MeOH}$

Right structure (Triacetyl-6-bromo-β-methylglycoside): CHOMe—, CHOAc, CHOAc, CHOAc, CH—, CH₂Br

Glycoseens. The glycoseens differ from the anhydro sugars in that a double bond is formed during the elimination of the elements of water from the aldoses. The two best-known types are those with the double bond between carbons one and two, or between carbons five and six. The *1,2-glycoseens* are prepared [37] by reacting the acetobromo sugar (I) with diethylamine, whereby hydrogen bromide is eliminated and tetra-acetyl-1,2-glycoseen (II) is formed.

Structures:

I: CHBr—, CHOAc, CHOAc, CHOAc, CH—, CH₂OAc (with O)

→

II: CH=, COAc, CHOAc, CHOAc, CH—, CH₂OAc (with O)

→

III: CH=, COH, CHOH, CHOH, CH—, CH₂OH (with O)

→

IV: CH₂, CO, CHOH, CHOH, CH—, CH₂OH (with O)
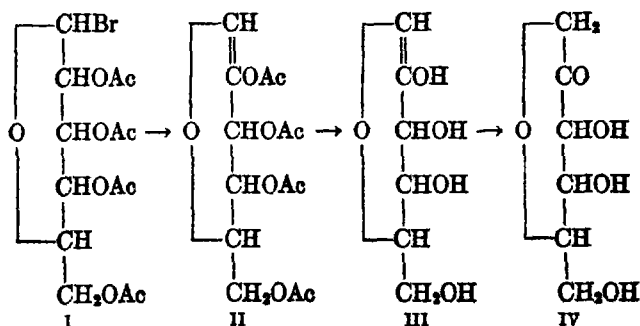
This will be seen to be the acetate of an enolized 1,5-anhydro-2-ketohexose (III) and might be expected to revert to the keto form (IV) on deacetylation. Actually, on attempted deacetylation no definite substance has been secured, and reacetylation of the product thus obtained does not give the original acetylglucoseen. With phenylhydrazine, however, the product is apparently

$$
\overset{\textstyle\frown}{CH_2C(=NNHC_6H_5)C(=NNHC_6H_5)CHOHCHCH_2OH}
$$

(with O bridge)

[37] Maurer and Mahn, *Ber.*, **60**, 1316 (1927); Maurer, *Ber.*, **62**, 332 (1929).

which is the "osazone" of IV.[38] It is of interest that treatment of tetraacetyl-1,2-glucoseen with excess alkali, and back titration with acid, as in an acetyl determination, uses five and not four equivalents of alkali.

Tetraacetyl-1,2-glucoseen reacts with chlorine, giving a crystalline compound which decomposes spontaneously. However, the chlorine may be removed by immediate hydrolysis with moist silver carbonate and there then results the acetate of glucosone hydrate.[39]
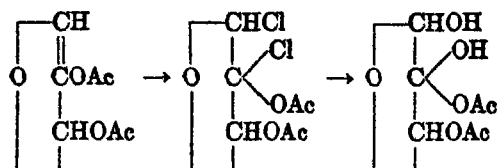
This acetate is readily converted with acetic anhydride and pyridine to diacetylkojic acid. Owing to loss of configuration this same product may be similarly prepared from tetraacetyl-1,2-galactoseen (through galactosone hydrate).

Tetraacetylglucosone hydrate   Diacetylkojic acid   Tetraacetylgalactosone hydrate
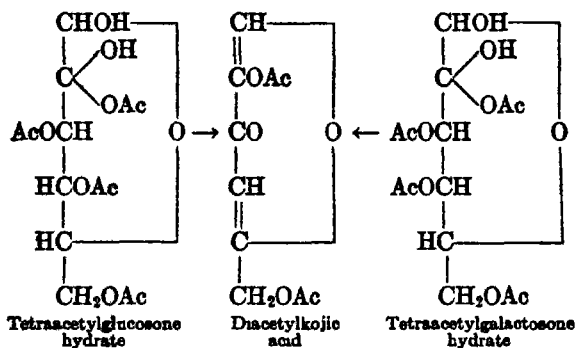
Kojic acid is of biological interest as it is formed in appreciable amounts by the action of many molds not only upon hexoses but also upon pentoses, trioses, glycerol, and other substances.

Tetraacetyl-1,2-glucoseen has been used by Zervas to establish the structure of styracitol. Hydrogenation of the glucoseen, using a palladium catalyst, and hydrolysis of the resultant tetraacetyl derivative produced styracitol which was therefore assumed [40] to be 1,5-anhydrosorbitol. However, the configuration of carbon atom two is not established by this synthesis, and later evidence [41, 42] indicates styracitol to be 1,5-anhydromannitol.

[38] Bergmann and Zervas, *Ber.*, **64**, 1434, 2032 (1931).
[39] Maurer, *Ber.*, **63**, 25 (1930); Maurer and Müller, *Ber.*, **63**, 2069 (1930).
[39] Zervas, *Ber.*, **63**, 1689 (1930).
[41] Freudenberg and Rogers, *J. Am. Chem. Soc.*, **59**, 1602 (1937).
[42] Zervas and Papadimitriace, *Ber.*, **73**, 174 (1940).

CH
‖
COAc
|
CHOAc   (H) → O
|
CHOAc   (Pd)
|
CH
|
CH₂OAc

Tetraacetyl-1,2-glucoseen

CH₂
|
CHOAc
|
CHOAc   Hydrolysis →
|
CHOAc
|
CH
|
CH₂OAc

Dihydrotetraacetylglucoseen

CH₂
|
CHOH
|
CHOH
|
CHOH
|
CH
|
CH₂OH

Styracitol
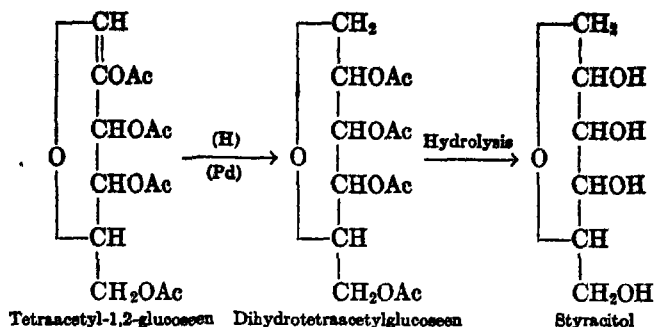
Related to 1,2-glucoseen is *5,6-glucoseen*. This is most easily prepared by reacting the 6-bromo or 6-iodo compound with silver fluoride in pyridine solution although it is also one of the products of the reaction of 6-tosyl*iso*diacetoneglucose and alcoholic ammonia.[43]

CHO〉C(CH₃)₂
CHO〉
O CHO〉C(CH₃)₂   NH₂ →
CH〉
CHO〉C(CH₃)₂
CH₂OTls

6-Tosyl*iso*-
diacetoneglucose

CHO〉C(CH₃)₂
CHO〉
O CHO
CH〉C(CH₃)₂
CO
CH₂

*Iso*diacetone-
5,6-glucoseen

CHOMe
CHOAc
O CHOAc   AgF
CHOAc   C₅H₅N
CH
CH₂X

6-Halogeno-
methylglucoside

CHOMe
CHOH
→ O CHOH
CHOH
C
CH₂

Methyl-5,6-
glucoseenide
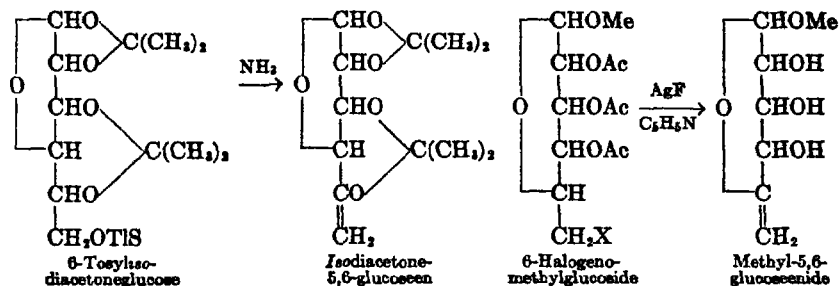
Like the 1,2-glucoseen it may be considered to be an enol, stabilized by the internal ether ring. Upon acid hydrolysis the ring is destroyed and the product ketonizes, giving a 5-keto-6-desoxyhexose, isorhamnonose. Similarly, diacetone-5,6-galactoseen on acid hydrolysis gives the isomeric fuconose.[44]

CH₃OCH—
|
HCOH
|
HOCH
|
HCOH   O   AgF
| C₅H₅N
HC
|
CH₂X

6-Halogeno-
methylglucoside

CH₃OCH—
|
HCOH
|
HOCH
|
HCOH   O   Acid
| hydrolysis
C
‖
CH₂

Methyl-5,6-
glucoseenide

CHO
|
HCOH
|
HOCH
|
HCOH
|
COH
‖
CH₂

5,6-Glucoseen

CHO
|
HCOH
|
HOCH
|
HCOH   →
|
CO
|
CH₃

Isorham-
nonose
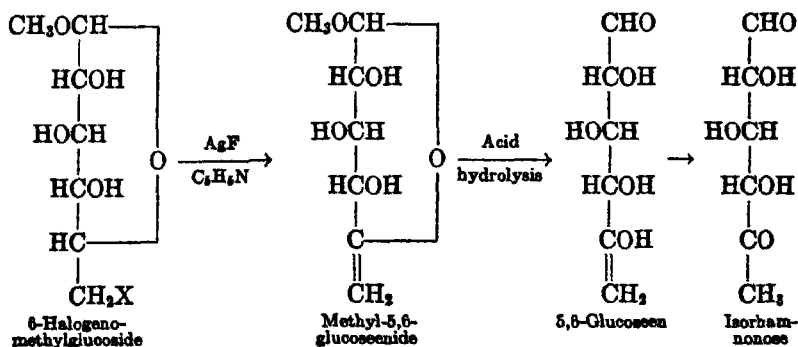
[43] Helferich and Himmen, *Ber.*, **61**, 1825 (1928); Ohle and v. Vargha, *Ber.*, **62**, 2425 (1929).

[44] Helferich and Himmen, *Ber.*, **62**, 2136 (1929); Ohle and Deplanque, *Ber.*, **66**, 12 (1933).

```
  ┌────────CHO                      CHO        CHO
  │         │  ╲CMe₂                 │          │
  │        HCO  ╱                   HCOH       HCOH
  │         │                        │          │
 O         OCH              Acid    HOCH       HOCH
     Me₂C  │        ────────────→    │    →     │
  │        OCH             hydrolysis HOCH       HOCH
  │         │                        │          │
  └─────────C                       COH        CO
            ‖                        ‖          │
           CH₂                      CH₂        CH₃
   Diacetone-5,6-galactoseen      5,6-Galactoseen  Fuconose
```
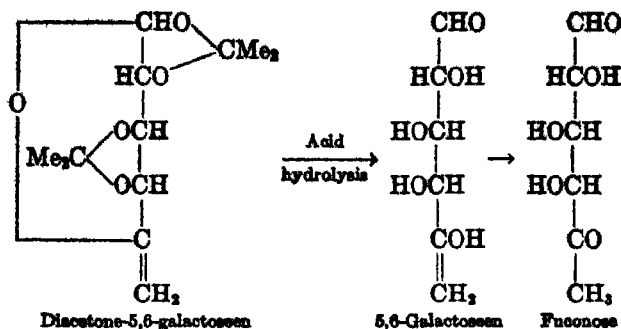
In addition to the intrinsic interest of a reaction of this type, a further significance attaches to it in connection with the alkaline rearrangements which will be considered later and which involve internal oxidations and reductions. It was also recently suggested [45] that this reaction might be used to distinguish between furanohexosides and pyranohexosides as the former should give a keto compound immediately

```
     └─CH        └─CH        └─CH
        │           │           │
      CHOH  →     COH   →     CO
        │           ‖           │
      CH₂X         CH₂         CH₃
```
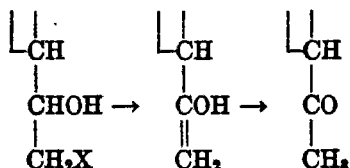
whereas the latter could do so only after hydrolysis, as indicated above. Experimentally, however, it was found that appropriate furanosides of both mannitol and glucose [46] failed to react with silver fluoride in pyridine, and no further evidence along this line has been obtained.

A final reaction of 5,6-glucoseen which may be noted is its oxidation with lead tetraacetate [47] to a derivative which hydrolyzes in water and gives a 5-ketohexose:

```
  ┌─CHOMe        ┌─CHOMe       CHO
  │   │          │   │          │
  │  CHOAc       │  CHOAc      CHOAc
  │   │          │   │          │
 O  CHOAc  →    O  CHOAc  →    CHOAc
  │   │          │   │          │
  │  CHOAc       │  CHOAc      CHOAc
  │   │          │   │          │
  └──C           └──COAc       CO
     ‖                          │
    CH₂          CH₂OAc        CH₂OAc
 Triacetyl-5,6-methyl-                  Tetraacetyl-
   glucoseenide                         5-ketohexose
```
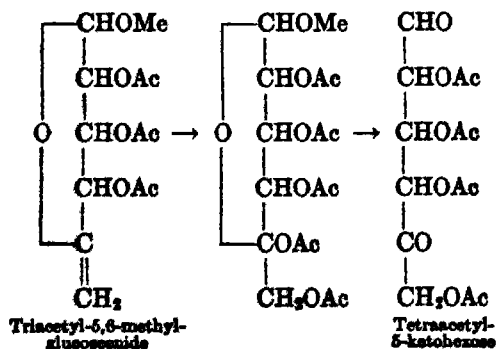
[45] Müller, Ber., 65, 1051 (1932).
[46] Helferich and Lang, J. prakt. Chem., 132, 321 (1932).
[47] Helferich and Bigelow, Z. physiol. Chem., 200, 263 (1931).

Evidence for the configuration of styracitol was obtained[42] by its conversion to what might be considered a 5,6-glycoseen. Styracitol was converted, through the tosyl compound, to the tribenzoyl-6-iodo derivative. This was unsaturated with silver fluoride in pyridine, and the resultant product was oxidized with lead tetraacetate, analogously to the reactions above. Hydrolysis with sodium methoxide yielded d-fructose, isolated as the osazone. This establishes the configuration of styracitol as being that of mannose rather than of glucose.

Styracitol

d-Fructose
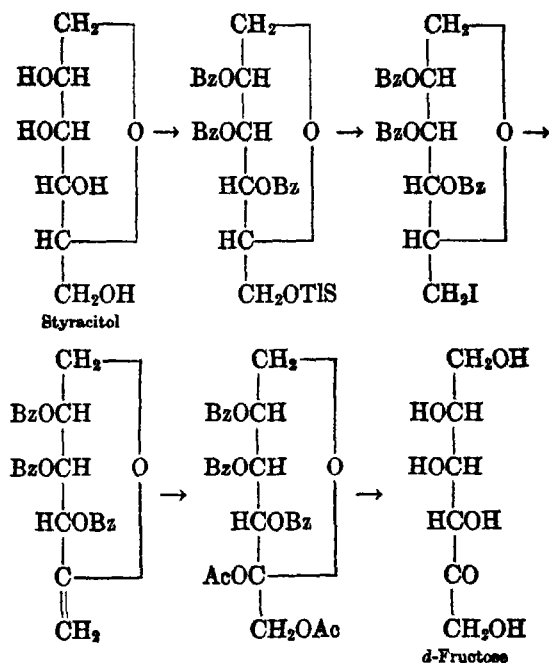
In addition to the 1,2- and 5,6-glycoseens, one example of the *3,4-glycoseens* has been described. This was formed as a by-product in the reaction of 3-tosyldiacetoneglucose with hydrazine. Although its structure has not been confirmed, it appears to be diacetone-3,4-glucoseen,
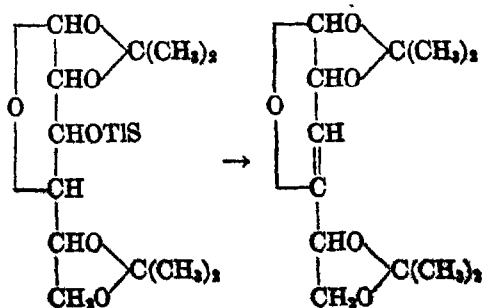
thus providing an additional example of this interesting group of substances.

Glycals. The glycals are unsaturated derivatives, formed by reduction of the acetobromo compounds with zinc in acetic acid, followed by hydrolysis of the acetyl groups.[48] They differ from the parent aldose in having lost an oxygen atom and the elements of water, while a double bond has appeared.

$$
\begin{array}{ccc}
\overset{\displaystyle |}{\text{O}}\begin{array}{l}
\text{--CHBr} \\
\text{CHOAc} \\
\text{CHOAc} \\
\text{CHOAc} \\
\text{--CH} \\
\text{CH}_2\text{OAc}
\end{array}
&
\overset{\displaystyle |}{\text{O}}\begin{array}{l}
\text{--CH} \\
\text{CH} \\
\text{CHOAc} \\
\text{CHOAc} \\
\text{--CH} \\
\text{CH}_2\text{OAc}
\end{array}
&
\overset{\displaystyle |}{\text{O}}\begin{array}{l}
\text{--CH} \\
\text{CH} \\
\text{CHOH} \\
\text{CHOH} \\
\text{--CH} \\
\text{CH}_2\text{OH}
\end{array}
\end{array}
$$

Bromo-tetraacetylhexose → Triacetylglycal → Glycal
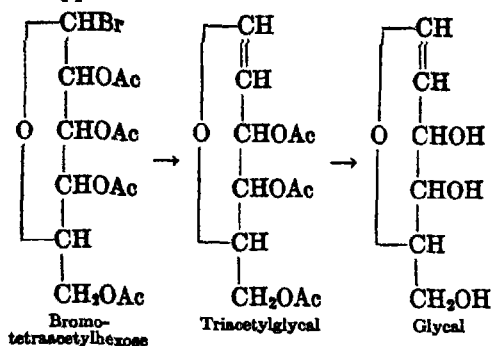
With glucose, it has been shown that this series of changes is unaccompanied by a shift in ring,[49] for methylation of glucal, oxidation with perbenzoic acid, further methylation, and hydrolysis give ordinary tetramethylglucopyranose (p. 1555).

Glucal → Trimethyl-glucal → Trimethyl-g'ucose → Pentamethyl-glucose → Tetramethyl-glucopyranose

(CH=CH, O, CHOH, CHOH, CH, CH₂OH) → (CH=CH, O, CHOMe, CHOMe, CH, CH₂OMe) → (CHOH, CHOH, O, CHOMe, CHOMe, CH, CH₂OMe) → (CHOMe, CHOMe, O, CHOMe, CHOMe, CH, CH₂OMe) → (CHOH, CHOMe, O, CHOMe, CHOMe, CH, CH₂OMe)
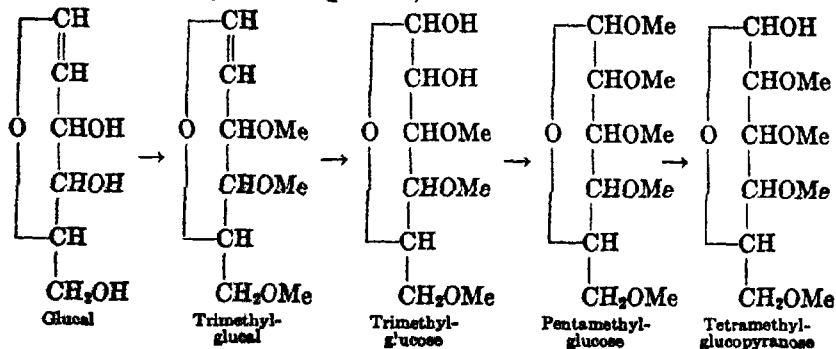
The oxidation of the glycals with perbenzoic acid is one of the most interesting reactions of this group of compounds. Apparently a mixture of the two epimers is invariably formed:

$$
\overset{\displaystyle |}{\text{O}}\begin{array}{l}
\text{--CH} \\
\text{CH} \\
\text{CHOH}
\end{array}
\;\rightarrow\;
\overset{\displaystyle |}{\text{O}}\begin{array}{l}
\text{--CHOH} \\
\text{HCOH} \\
\text{CHOH}
\end{array}
\;+\;
\overset{\displaystyle |}{\text{O}}\begin{array}{l}
\text{--CHOH} \\
\text{HOCH} \\
\text{CHOH}
\end{array}
$$

---
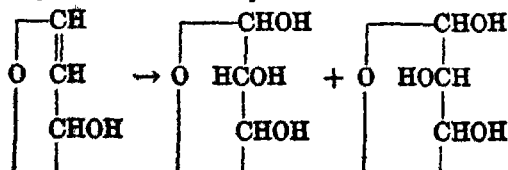
[48] Fischer, *Ber.*, **47**, 196 (1914); Bergmann and Schotte, *Ber.*, **54**, 446 (1921); Bergmann and Freudenberg, *Ber.*, **62**, 2783 (1929).

[49] Hirst and Woolvin, *J. Chem. Soc.*, 1131 (1931).

but the proportions of the two may vary widely,[50] and the conditioning factors are not well understood. Thus with glucal and rhamnal, the products are almost exclusively mannose and rhamnose, whereas with galactal, although talose predominates, much galactose is formed. Moreover, substitution of glucal in position three may change the proportions enormously, for the glucose derivative predominates on oxidation of triacetylglucal and 3-methylglucal, and trimethylglucose is the principal or perhaps only product from trimethylglucal.

The mechanism of this oxidation with perbenzoic acid has not been fully established. As mentioned, the oxidation of glucal in the presence of moisture leads to the production of mannose. In the absence of moisture, however, if the intermediate product is treated with methyl alcohol, α-methylmannoside is formed, and in similar fashion α-methylrhamnoside is formed from rhamnal. This indicates the intermediate occurrence of a 1,2-anhydro sugar, but isolation of a compound of this nature from the reaction mixture has not yet been achieved.
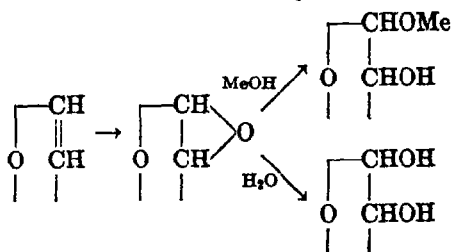
It might be expected that triacetylglucal (I) with perbenzoic acid would give a compound identical with that (II) prepared by Brigl (see 1,2-anhydro sugars, p. 1618).
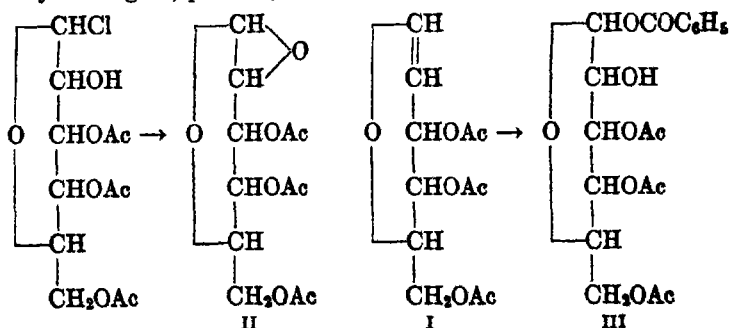
Interestingly, however, the major substance which is isolated from this oxidation is 1-benzoyl-3,4,6-triacetylglucose (III), although other glucose and mannose derivatives are simultaneously formed.

Other reactions of the glycals have to do with their isomerization. Thus when triacetylglucal is boiled with water, diacetylpseudoglucal is formed, and this, on hydrolysis with barium hydroxide solution, by an elaborate rearrangement gives isoglucal, and in lesser amount, protoglucal.[51]

| CH | CHOH | CH₂ | CHO |
| Triacetylglucal | Diacetylpseudoglucal | Isoglucal | Protoglucal |
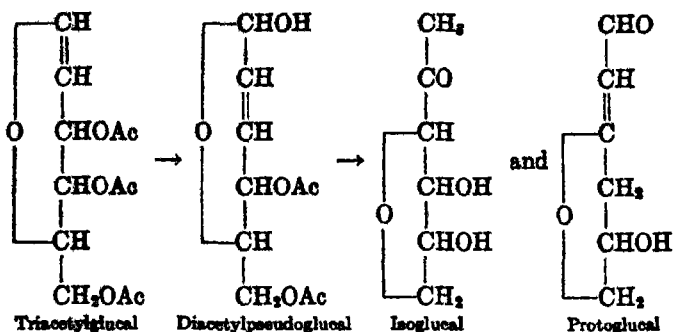
Protoglucal reduces Fehling's solution, and its presence in traces is probably responsible for the original erroneous report that glucal is a reducing substance.

The glycals may be oxidized by ozone to give the corresponding aldose of one less carbon atom, which incidentally proves the position of the double bond, or they may be hydrogenated to the hydroglycals. Halogens may be added to triacetylglucal to give a mixture of two epimeric acetohalogeno-2-halogenohexoses which have proved useful for certain syntheses.
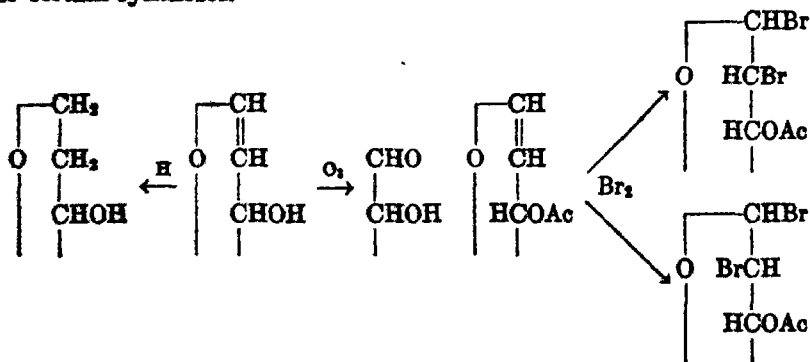
The addition product with hydrogen bromide appears to have the bromine in the 2-position instead of in the 1-position.[52]

[51] Bergmann and Freudenberg, *Ber.*, **64**, 158 (1931).
[52] Fischer, Bergmann, and Schotte, *Ber.*, **53**, 517 (1920).

$$
\begin{array}{cc}
\begin{array}{l}
\text{┌─CH} \\
\text{|} \quad \text{‖} \\
\text{O} \quad \text{CH} \\
\text{|} \quad \text{|} \\
\text{|} \quad \text{CHOAc} \\
\text{|} \quad \text{|}
\end{array}
& \xrightarrow{}
\begin{array}{l}
\text{┌─CH}_2 \\
\text{|} \quad \text{|} \\
\text{O} \quad \text{CHBr} \\
\text{|} \quad \text{|} \\
\text{|} \quad \text{CHOAc} \\
\text{|} \quad \text{|}
\end{array}
\end{array}
$$
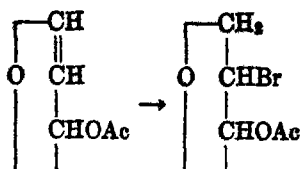
This is unfortunate as otherwise the acetobromo derivatives of the 2-desoxy sugars would be easily available, and should prove useful for synthetic work.

Desoxy Sugars. This series owes its name to the fact that one or more CHOH groups have been deprived of an oxygen atom and converted into CH_2. Desoxy sugars from several natural sources have been described; of the *2-desoxy sugars*, the only one which occurs naturally is 2-desoxyribose (arabinose), which has been shown to be the sugar component of thymus nucleic acid. The synthetic preparation of the series has been achieved from the glycals in two ways. In one of these the 2-halogenoaldoses described above are reduced, and in the other water is added directly to the glycal, usually with sulfuric acid as catalyst, as indicated: [63]

$$
\begin{array}{l}
\text{┌─CHOH} \\
\text{|} \quad \text{|} \\
\text{O} \quad \text{CH}_2 \\
\text{|} \quad \text{|} \\
\text{|} \quad \text{CHOH} \\
\text{|} \quad \text{|}
\end{array}
\xleftarrow[\text{(Acid)}]{H_2O}
\begin{array}{l}
\text{┌─CH} \\
\text{|} \quad \text{‖} \\
\text{O} \quad \text{CH} \\
\text{|} \quad \text{|} \\
\text{|} \quad \text{CHOH} \\
\text{|} \quad \text{|}
\end{array}
\xrightarrow{Br_2}
\begin{array}{l}
\text{┌─CHBr} \\
\text{|} \quad \text{|} \\
\text{O} \quad \text{CHBr} \\
\text{|} \quad \text{|} \\
\text{|} \quad \text{CHOH} \\
\text{|} \quad \text{|}
\end{array}
\xrightarrow[Ag_2O]{CH_3OH}
\begin{array}{l}
\text{┌─CHOCH}_3 \\
\text{|} \quad \text{|} \\
\text{O} \quad \text{CHBr} \\
\text{|} \quad \text{|} \\
\text{|} \quad \text{CHOH} \\
\text{|} \quad \text{|}
\end{array}
\xrightarrow{(H)}
\begin{array}{l}
\text{┌─CHOCH}_3 \\
\text{|} \quad \text{|} \\
\text{O} \quad \text{CH}_2 \\
\text{|} \quad \text{|} \\
\text{|} \quad \text{CHOH} \\
\text{|} \quad \text{|}
\end{array}
$$
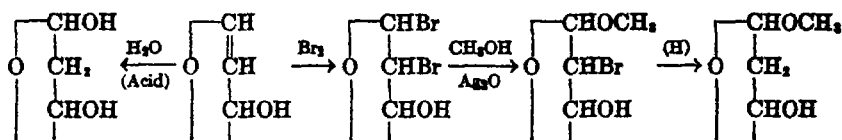
An interesting method for preparing the 2-desoxygluconic acids is by means of an intramolecular oxidation and reduction which occurs when 2-chloroglucose (triacetyl- or trimethyl-) is heated with lead hydroxide: [64]

$$
\text{RCHClCHO} \xrightarrow[H_2O]{Pb(OH)_2} \text{RCH}_2\text{COOH}
$$

Triacetyl-2-chloroglucose $\qquad$ 2-Desoxygluconic acid
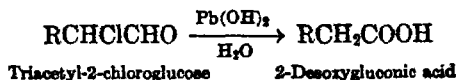
A characteristic of the 2-desoxy sugars is their great reactivity. The pyranosides in this series, for example, are sometimes formed or hydrolyzed as rapidly as the furanosides of the ordinary sugars. Thus far only one 1-bromo derivative has been prepared. The ready formation of levulinic acid from the 2-desoxypentoses on treatment with acid will be discussed later (p. 1638).

A single *3-desoxy* sugar has been described as resulting from the

[63] Bergmann, Schotte, and Leschinsky, *Ber.*, **56**, 1052 (1923); Gehrke and Aichner, *Ber.*, **60**, 918 (1927); Levene and Mori, *J. Biol. Chem.*, **83**, 803 (1929); Levene, Mikeska, and Mori, *ibid.*, **85**, 785 (1930).

[64] Danilov and Gakhokidze, *J. Gen. Chem. (U.S.S.R.)*, **6**, 704 (1936).

catalytic reduction of diacetone-3,4-glucoseen, but these structures have not been well authenticated.

$$
\begin{array}{ccc}
\text{Diacetone-} & \text{Diacetone-} & \text{Diacetone-} \\
\text{glucose} & \text{3,4-glucoseen} & \text{3-desoxyglucose}
\end{array}
$$
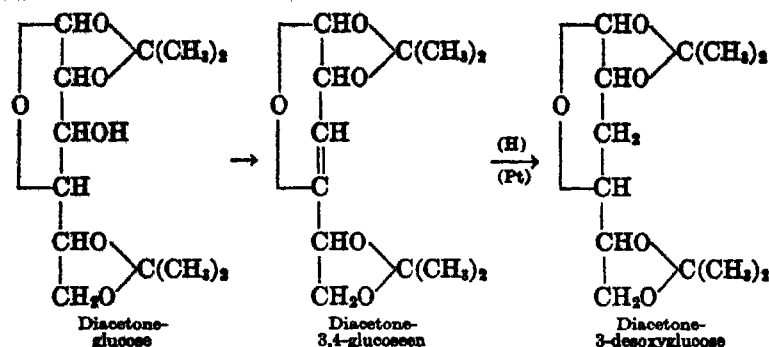
A further series of sugars very widely distributed in nature are the methyloses in which the terminal group is the desoxy. Although much work has been done in this series, it seems sufficient to indicate the synthetic method of their preparation, which is the reduction of a halogen attached to the terminal carbon. This is conveniently the iodide, introduced by replacing the tosyl group, although other halogens have been similarly reduced.

As a typical example of a synthesis in this series the preparation of *d*-xylomethylose [55] may be cited:

$$
\begin{array}{cccc}
\text{Xylose} & \text{Monoacetone-} & \text{5-Tosyl-} \\
 & \text{xylose} & \text{monoace-} \\
 & & \text{tonexylose}
\end{array}
$$

$$
\begin{array}{ccc}
\text{5-Iodo-} & \text{Monoacetone-} & \text{Xylo-} \\
\text{monoace-} & \text{xylomethylose} & \text{methylose} \\
\text{tonexylose} & &
\end{array}
$$
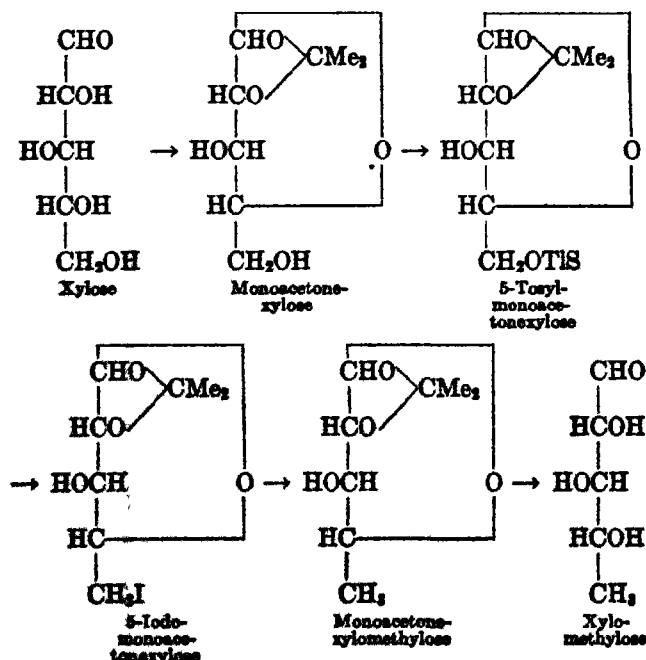
It is possible, of course, to have more than one carbon as a desoxy group, and the hydrogenation products of the glycals afford example: of this type of compound. Thus dihydroglucal is really 2-desoxystyraci-tol, and dihydropseudoglucal is 2,3-bisdesoxyglucose.

$$
\begin{array}{cccc}
\left[\begin{matrix} \text{CH} \\ \| \\ \text{CH} \\ | \\ O \ \text{CHOH} \\ | \\ \text{CHOH} \\ | \\ \text{CH} \\ | \\ \text{CH}_2\text{OH} \end{matrix}\right]
&
\left[\begin{matrix} \text{CH}_2 \\ | \\ \text{CH}_2 \\ | \\ O \ \text{CHOH} \\ | \\ \text{CHOH} \\ | \\ \text{CH} \\ | \\ \text{CH}_2\text{OH} \end{matrix}\right]
&
\left[\begin{matrix} \text{CHOH} \\ | \\ \text{CH} \\ \| \\ O \ \text{CH} \\ | \\ \text{CHOAc} \\ | \\ \text{CH} \\ | \\ \text{CH}_2\text{OAc} \end{matrix}\right]
&
\left[\begin{matrix} \text{CHOH} \\ | \\ \text{CH}_2 \\ | \\ O \ \text{CH}_2 \\ | \\ \text{CHOAc} \\ | \\ \text{CH} \\ | \\ \text{CH}_2\text{OAc} \end{matrix}\right]
\end{array}
$$

Glucal   2-Desoxy-   Diacetyl-   Diacetyl-
          styracitol   pseudoglucal 2,3-bisdesoxyglucose
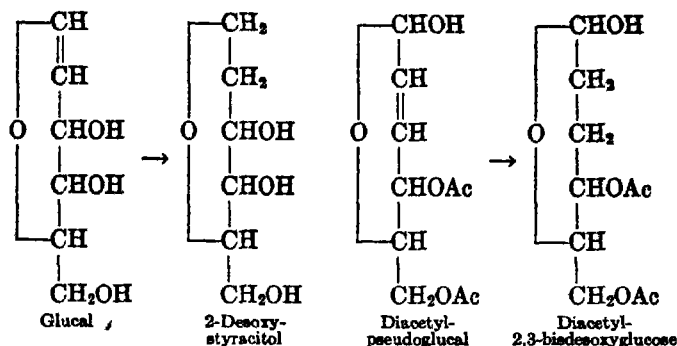
The naturally occurring digitoxose is, in fact, a bisdesoxy sugar 2-desoxyallomethylose (or 2,6-bisdesoxyallose), and cymarose is it: 3-methyl ether.[56] Sarmentose and digitalose are another pair which occu: as components of the cardiac glycosides.

Ascorbic Acid—Vitamin C. The rapid and successful solution o' the ascorbic acid problem, both as to structure and synthesis, constitute: one of the major achievements in the field of sugar chemistry, and o: this account is deserving of special consideration. Inclusion at thi: point is justified by the fact that the vitamin is an unsaturated suga: derivative, and because the problems connected with it give valuabl: information in the field of these unsaturated derivatives.

Szent-Györgyi had isolated from a number of vegetable and plan: sources, as well as from suprarenal cortex, a "hexuronic acid," whicl he considered to be associated with respiratory processes. Moreover he had pointed out that the distribution of this substance paralle: that of vitamin C. Although Zilva claimed that there was no constan' relationship between the antiscorbutic activity and the reducing prop erty which was a characteristic of Szent-Györgyi's acid, this claim wa: disputed some years later by Tillmans and co-workers, who found tha such a relationship did in fact exist. Szent-Györgyi and his co-worker: next demonstrated for their crystalline material a definite antiscorbuti activity, and King with his collaborators independently and simultane ously described a crystalline vitamin C preparation which had all th: physical and chemical properties of the "hexuronic acid."[57] Afte

[56] Windaus and Hermanns, *Ber.*, **48**, 993 (1915); Elderfield, *J. Biol. Chem.*, **111**, 5: (1935).

[57] For review of literature see *Annual Review of Biochemistry*, Stanford University Pres Stanford University, Calif. (1934), Vol. II, chapter on vitamins.

further investigation it was at length agreed that the two substances were identical. Szent-Györgyi was finally able to secure relatively large amounts of the acid from Hungarian paprika, and it was thus made available for study. Intensive investigations were simultaneously undertaken in a number of laboratories, and in a surprisingly short time the entire problem was solved.

"Hexuronic acid," or ascorbic acid as it was soon called, behaves chemically like an unsaturated monobasic acid. It has the empirical formula $C_6H_8O_6$ and contains one double bond. It gives a dimethyl derivative with diazomethane, which is a specific reagent for methylation of acidic hydrogens. These facts are all adequately explained on the basis of the earlier formulas, given below, in which one of the acidic hydrogens is that of the carboxyl group and the other is that of the tautomeric hydroxyl:

```
COOH      COOH       COOH       COOH      COOH        COOH       COOE
 |         |          |          |         |           |          |
CO       'CO       ┌─C         CO        COH       ┌─COH      ┌─COH
 |         |       │  ‖         |         ‖         │  |       │  |
CO        COH    O │ COH        CH₂       CH       O│ CH      O│ CH
 |         |       │  |          |         |        │  |        │  ‖
CH₂  ⇌    CH       │ CHOH        CO   ⇌   CO        │ COH      │ COH
 |         |       │  |          |         |        │  |        │  |
CHOH      CHOH    └─CH         CHOH      CHOH      └─CH       CHOE
 |         |          |          |         |           |          |
CH₂OH     CH₂OH     CH₂OH       CH₂OH     CH₂OH      CH₂OH      CH₂
```
Cox, Hirst, and   Micheel   Karrer et al.
   Reynolds   and Kraft
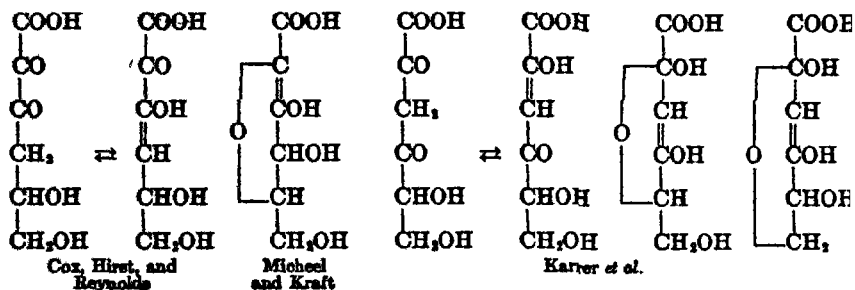
Hirst, however, made the significant observation that the dimethyl derivative dissolved in alkali without splitting off a methyl group,[55] and was thus led to the formula which is now accepted as correct:

```
        CO──┐
        │   │
      HOC   │
        ‖   │ O
      HOC   │
        │   │
       CH───┘
        │
      HOCH
        │
      CH₂OH
```
Ascorbic acid
(2,3-Enediol-l-gulono-1,4-lactone)
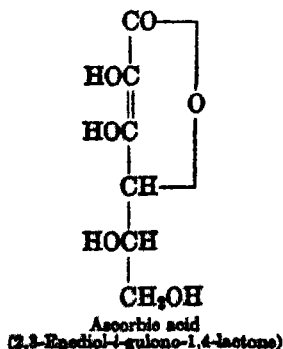
The configuration was deduced from that of the oxidation product of ascorbic acid, which was shown to be 2,3-diketo-l-gulonic acid, as well as from the fact that ozonization of ascorbic acid and of its methyl deriva-

[55] Hirst, Chemistry & Industry, **52**, 221 (1933).

tives gave *l*-threonic acid and its methyl ethers. On the basis of this formula, ascorbic "acid" is a highly stable *lactone*, which owes its acidic properties entirely to an enolized keto group. It is of interest that, whereas ozonization of tetramethylascorbic acid, prepared by further methylation of crude dimethylascorbic acid, gives a mixture of 3,4-dimethyl-*l*-threonic acid and the epimeric 3,4-dimethyl-*l*-erythronic acid, if the dimethyl derivative is first isolated in crystalline form and then further methylated and ozonized, only the threonic derivative is secured. This is apparently due to further tautomerizations involving the fourth carbon atom.

That the formula above was in fact correct was soon confirmed by a series of brilliant syntheses, of which the first was that of Reichstein, Grüssner, and Oppenauer.[59] These authors started with the osone of *d*-xylose, and by addition of hydrogen cyanide, followed by hydrolysis, effected the synthesis of the enantiomorphic *d*-ascorbic acid. This method was then utilized by Haworth and co-workers,[60] who started with *l*-xylose and with somewhat modified procedure effected the synthesis of the naturally occurring *l*-ascorbic acid. Further studies on this method have revealed that the mechanism is apparently rather complex, the changes probably being as follows:
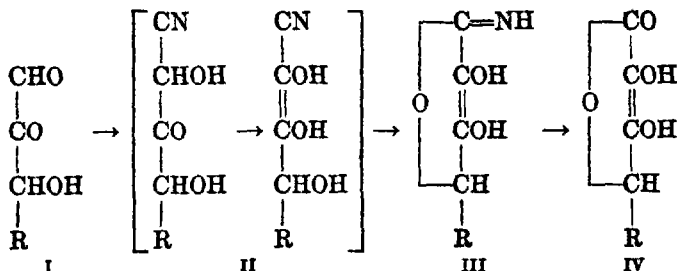
The first intermediate isolated is the crystalline cyclic imino compound (III), and this, on treatment with acid, passes smoothly into the ascorbic acid (IV). This type of synthesis is general, and a number of isomeric "ascorbic acids" have been prepared in this manner.

The second important method of synthesis of ascorbic acid is also due to Reichstein,[61] who found that *l*-sorbose (prepared from *d*-sorbitol by the action of *Acetobacter xylinum*; and the *d*-sorbitol from *d*-glucose by catalytic reduction) forms a 2,3,4,6-diacetone derivative which on

[59] Reichstein, Grüssner, and Oppenauer, *Helv. Chim. Acta*, 16, 561, 1019 (1933); 17, 510 (1934). See, also, Haworth *et al.*, *J. Chem. Soc.*, 1419 (1933); 62, 1192 (1934).

[60] Ault *et al.*, *J. Chem. Soc.*, 1419 (1933).

[61] Reichstein and Grüssner, *Helv. Chim. Acta*, 17, 311 (1934).

alkaline oxidation yields diacetone-2-keto-*l*-gulonic acid. Removal of the acetone groups by acid hydrolysis gives the free acid, and this on heating with water is transformed into ascorbic acid. A somewhat better preparation consists in converting the free acid into its methyl ester and heating this with sodium methoxide in methyl alcohol, whereby the sodium salt of *l*-ascorbic acid is secured.

CH$_2$OH	CH$_2$OH	CH$_2$OH
HOCH	HOCH	CO
HOCH	HOCH	HOCH
HCOH →	HCOH →	HCOH
HOCH	HOCH	HOCH
CHO	CH$_2$OH	CH$_2$OH
d-Glucose	*d*-Sorbitol	*l*-Sorbose

Diacetone-*l*-sorbose   2-Keto-*l*-gulonic acid   *l*-Ascorbic acid
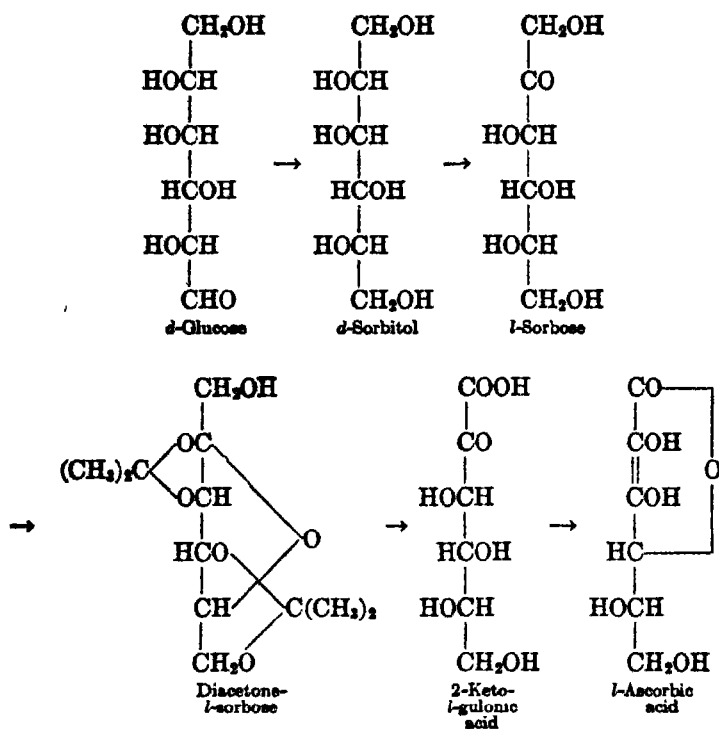
The 2-keto-*l*-gulonic acid, which is an intermediate in this synthesis, was obtained more easily by Haworth,[62] who has found that direct oxidation of ketoses with nitric acid leads to preferential oxidation of the primary alcoholic group adjacent to the keto group. In this manner ascorbic acid was easily prepared from *l*-sorbose, and *d*-araboascorbic acid (the nomenclature refers the ascorbic acid to the parent aldose of one less carbon) from *d*-fructose.

A totally different method of synthesis is due to Helferich and Peters.[63] Either aldoses or acetylated cyanohydrins may be employed,

[62] Haworth, *Nature*, **134**, 724 (1934); *Brit. Assoc. Advancement Sci. Rept.*, 295 (1934)
[63] Helferich and Peters, *Ber.*, **70**, 465 (1937).

and they are treated with glyoxylic esters in alkaline solution. The
method is novel in that the sugar chain is increased by two carbons:

$$
\begin{array}{ccc}
 & COOC_2H_5 & COOH \\
 & | & | \\
CN & CHO & C-OH \\
| & + & \| \\
CHOAc \rightarrow & CHO & \rightarrow C-OH \\
| & | & | \\
CHOAc & CHOH & CHOH \\
| & | & | \\
R & R & R
\end{array}
$$
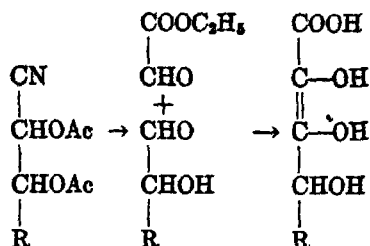
A series of synthetic analogs has been obtained by these methods,
and their physiological activity has been examined. Those prepared
include the only possible four-carbon analog, one of the two possible
five-carbon forms, all four of the possible six-carbon acids, and three of
the eight possible seven-carbon acids. Many other analogs have been
synthesized in an effort to correlate structure and biological activity. Of
those above, the synthetic l-ascorbic acid has the same activity as the
natural substance, as has its primary oxidation product; d-araboascorbic
acid has a slight activity, and none of the others may be regarded as
possessing any antiscorbutic activity: the 6-desoxy derivatives show
appreciable activity, however. The generalization, made simultane-
ously by Reichstein and by Haworth,[64] is that for antiscorbutic activity
the fourth carbon atom must be of the d-series. It is of some interest
that the imino compound (III), though closely related to ascorbic acid
chemically, is without activity.

Of further importance in this fascinating field are the interesting
substances reductone and reductic acid.[65]

$$
\text{CHOH=COHCHO} \qquad\qquad \text{HOC=C}\begin{array}{c}\diagup OH \\ | \quad\quad \diagdown CO \\ CH_2CH_2 \end{array}
$$

<center>Reductone   Reductic acid</center>
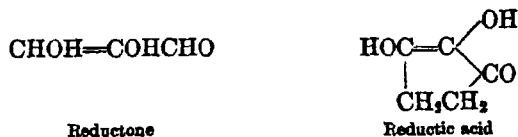
The first of these is the enol of hydroxymethylglyoxal, formed by action
of alkali on various carbohydrates; the second is formed by action of
dilute sulfuric acid at high temperatures. Both are characterized by
the same system of enediols as that in ascorbic acid, and like ascorbic
acid both reduce the characterizing indicator, dichlorophenolindo-

[64] Reichstein, *Nature*, **134**, 724 (1934); Haworth, *ibid.*, and *Brit. Assoc. Advancement Sci. Rept.*, 295 (1934).
[65] Norrish and Griffiths, *J. Chem. Soc.*, 2837 (1928); von Euler and Martius, *Svensk Kem. Tid.*, **45**, 73 (1933); Reichstein and Oppenauer, *Helv. Chim. Acta*, **16**, 988 (1933).

phenol. With additional knowledge it may be found that substances of this nature are of major importance in the chemical as well as in the biological degradations and transformations of the sugars.

ISOMERIZATIONS AND DEGRADATIONS

Acid Rearrangements. The mechanism of the reactions whereby the sugars are rearranged or are broken down into smaller fragments is of the greatest interest both chemically and biologically. It has, however, been the subject of so much research that only a brief outline of the conclusions is possible here. The simplest types of changes are effected by *acid* treatment, and the effect ranges from almost nothing with weak acids, to complex changes, eventuating in the formation of humic substances, with hot concentrated acids. Between these extremes lie simple conversions, produced by acids of intermediate concentration, such as the production of furfural from pentoses and the analogous production of methylfurfural from methylpentoses, and hydroxymethylfurfural and levulinic acid from hexoses:

```
CHO                CHO              CHO                CHO
|                  |                |                  |
CHOH             ┌─C              CHOH              ┌─C
|                │ ‖               |                │ ‖
CHOH    -3H₂O   │ CH     CHOH    -3H₂O   │ CH
|       ────►  O│               |       ────►  O│
CHOH             │ CH              CHOH              │ CH
|                │ ‖               |                │ ‖
CH₂OH            └─CH              CHOH              └─C
                                   |                   |
                                   CH₃                 CH₃
```

```
  CHO              CHO           HCOOH
┌─C               |               +              CHO
│ ‖               CHOH          COOH             |
│ CH    -3H₂O    |      -H₂O   |       -H₂O    CH₃
O│     ◄────    CHOH   ────►  CH₂    ◄────     |
│ CH              |               |              CHOH
│ ‖               CHOH          CH₂             |
└─C               |               |              CHOH
  |               CHOH          CO              |
  CH₂OH           |               |              CH₂OH
                  CH₂OH          CH₃
```
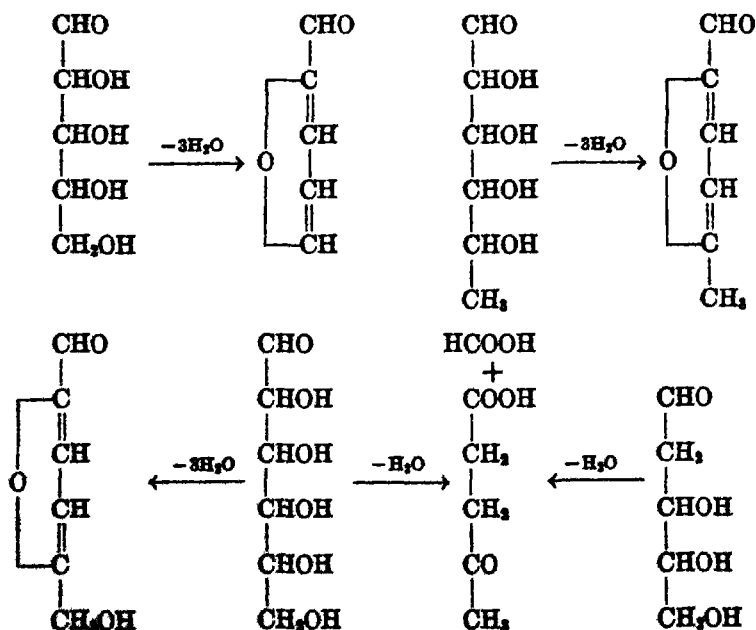
It will be observed that, with the exception of levulinic acid, all the products may be considered as being formed in identical fashion by abstraction of three molecules of water. Levulinic acid results on similar treatment of 2-desoxypentoses as well as of hexoses, and this

fact was for a long time responsible for the belief that the sugar of the thymus nucleic acids was a hexose. No very good evidence has been presented to account for the formation of levulinic acid, although it has been shown to result on acid treatment of hydroxymethylfurfural.[66]

$$
\begin{array}{c}
\text{CH} \!-\!\!-\!\! \text{CH} \\
\quad\Vert \qquad \Vert \\
\text{HOCH}_2\!-\!\text{CH} \qquad \text{C}\!-\!\text{CHO} \rightarrow \text{HCOOH} + \text{CH}_3\text{COCH}_2\text{CH}_2\text{COOH} \\
\diagdown\!\!\text{O}\diagup
\end{array}
$$
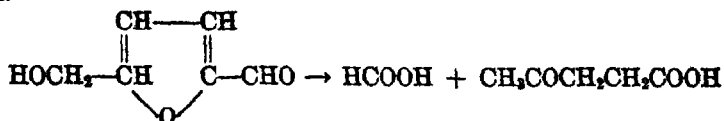
Levene and Mori have indicated the formation from 2-desoxypentoses in the following way:

$$
\begin{array}{llll}
\text{CHO} & \text{CHO} & \text{CHO} & \text{COOH} \\
| & | & | & | \\
\text{CH}_2 & \text{CH}_2 & \text{CHOH} & \text{CH}_2 \\
| & | & | & | \\
\text{CHOH} \rightarrow & \text{CHOH} \rightarrow & \text{CH}_2 & \rightarrow \text{CH}_2 \\
| & |\diagup^{\text{OH}} & | & | \\
\text{CHOH} & \text{C} & \text{CO} & \text{CO} \\
| & |\diagdown_{\text{OH}} & | & | \\
\text{CH}_2\text{OH} & \text{CH}_3 & \text{CH}_3 & \text{CH}_3
\end{array}
$$
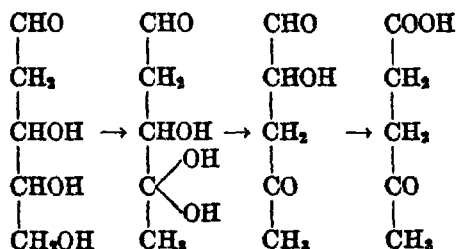
without attempting to substantiate this mechanism.

In connection with the formation of furfural, several observations may be mentioned. The first of these is the fact that the fully methylated pentoses, whether furanose or pyranose, give furfural on treatment with strong acid.[67] The yields in general are as high as those from the free pentoses and are sometimes higher. Moreover they appear to be independent of the furanose or pyranose ring form of the methylated sugar. Of interest in the same connection is the formation of methoxymethylfurfural from tetramethylfructofuranose. For this rearrangement Haworth has suggested the following mechanism, which assumes the enol as the first step:

$$
\begin{array}{llll}
\text{CH}_2\text{OMe} & \text{CHOMe} & \text{CHO} & \text{CHO} \\
| & \Vert & | & | \\
\lceil\text{COH} & \lceil\text{C} & \lceil\text{CH} & \lceil\text{C} \\
| & | & | & \Vert \\
\text{CHOMe} & \text{CHOMe} & \text{CHOMe} & \text{CH} \\
\text{O} \; | \rightarrow \text{O} \; | \rightarrow & \text{O} \; | \rightarrow & \text{O} \; | \\
\text{CHOMe} & \text{CHOMe} & \text{CHOMe} & \text{CH} \\
| & | & | & \Vert \\
\lfloor\text{CH} & \lfloor\text{CH} & \lfloor\text{CH} & \lfloor\text{C} \\
| & | & | & | \\
\text{CH}_2\text{OMe} & \text{CH}_2\text{OMe} & \text{CH}_2\text{OMe} & \text{CH}_2\text{OMe}
\end{array}
$$
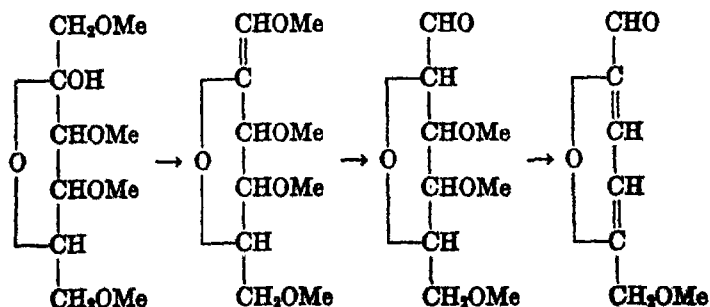
[66] Pummerer, Guyot, and Birkofer, Ber., 68, 480 (1935).
[67] Bott and Hirst, J. Chem. Soc., 2621 (1932).

Alkaline Rearrangements. Turning from the action of acids on sugars to that of *alkalies*, a much more complicated problem is encountered. For a logical treatment of the subject the effect of alkalies might be divided into, first, those changes which involve rearrangement without splitting, and, second, the changes involving scission of the molecule into smaller fragments. The first of these classes would include as its simplest case the problem of mutarotation or Walden inversion on the aldehydic carbon atom (which has been discussed, p. 1546), then epimerization or Walden inversion on the carbon adjacent to the aldehydic carbon, next the progressive wandering of the reducing group along the carbon chain, and finally such complicated rearrangements as those involved in saccharinic acid formation where branched-chain acids result. In the second of the above classes would be included the formation of aldehydic substances such as formaldehyde, acetaldehyde, and methylglyoxal, acids such as formic, acetic, lactic, and dihydroxybutyric, and finally reduced scission products such as alcohol. Unfortunately, however, in practice it becomes almost impossible to segregate these problems for separate discussion as each type of reaction is intimately concerned with each of the others, and deviation from such logical presentation becomes almost unavoidable.

In the study of epimerization, complex side reactions may be avoided by working with the sugar acids. By heating them with aqueous pyridine, apparently only the epimers are formed and the reaction, moreover, seems to be reversible. Both aldonic and saccharic acids exhibit this phenomenon as do the fully methylated γ- and δ-lactones.[48]

COOH	COOH		COOH	COOH
HCOH	HOCH		HCOH	HOCH
HOCH	HOCH	⇄	HOCH	HOCH
HCOH	HCOH		HOCH	HOCH
HCOH	HCOH		HCOH	HOCH
CH₂OH	CH₂OH		COOH	COOH
Gluconic acid	Mannonic acid		Mucic acid	Allomucic acid
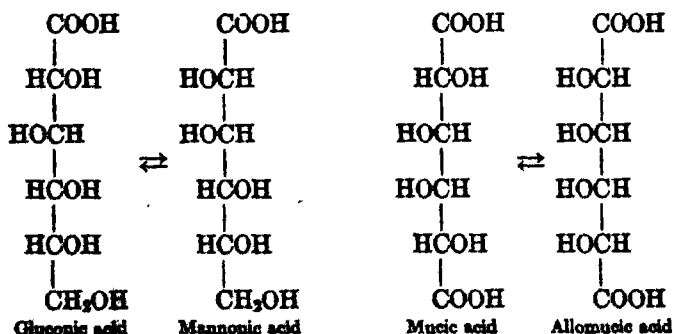
A similar simple inversion has been observed on treating certain of the methylated sugars with dilute alkali.

Not all reactions of this type are so simple, however, and if the free sugars are employed instead of the acids then the changes usually become

⁴⁸ Fischer, *Ber.*, **23**, 799 (1890); Haworth and Long, *J. Chem. Soc.*, 345 (1929); Hedenburg and Cretcher, *J. Am. Chem. Soc.*, **49**, 478 (1927).

much more complicated. In one of the simple cases, where glyceraldehyde is treated with anhydrous pyridine, a reversible conversion to dihydroxyacetone has been observed.

$$CHOCHOHCH_2OH \rightleftarrows CH_2OHCOCH_2OH$$
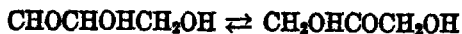
On treatment of glucose, on the other hand, with even as mild a reagent as saturated lime water, the first products are apparently mannose and fructose, but if the reaction is allowed to continue then a host of other products is formed. The nature of these substances and the mechanism of their formation have been extensively investigated, but they are still not fully understood. Nef, following Wohl, Lobry de Bruyn, and Alberda van Ekenstein, argued for the enol (p. 1584) as being the intermediate in the alkaline reactions of the carbohydrates, and did extensive research in corroboration of this idea.[69] This theory assumed the enol to be formed by alternate addition and removal of the elements of water, the change being considered progressive.

$$
\begin{array}{c}
\underset{\substack{|\\ \text{HCOH} \\ |}}{\text{CHO}}
\xrightarrow{+H_2O}
\underset{\substack{|\\ \text{HCOH} \\ |}}{\text{CH(OH)}_2}
\xrightarrow{-H_2O}
\underset{\substack{|\\ \text{COH} \\ |}}{\overset{\text{CHOH}}{\underset{}{\|}}}
\xrightarrow{+H_2O}
\underset{\substack{|\\ \text{HOCH} \\ |}}{\text{CH(OH)}_2}
\xrightarrow{-H_2O}
\underset{\substack{|\\ \text{HOCH} \\ |}}{\text{CHO}}
\end{array}
$$

$$\downarrow + H_2O$$

$$
\begin{array}{c}
\underset{\substack{|\\ \text{HCOH} \\ |}}{\overset{\text{CH}_2\text{OH}}{\underset{}{|}\text{CO}}}
\xleftarrow{-H_2O}
\underset{\substack{|\\ \text{HCOH} \\ |}}{\overset{\text{CH}_2\text{OH}}{\underset{}{|}\text{C(OH)}_2}}
\xrightarrow{-H_2O}
\underset{\substack{|\\ \text{COH} \\ |}}{\overset{\text{CH}_2\text{OH}}{\underset{}{\|}\text{COH}}}
\quad \text{etc.}
\end{array}
$$
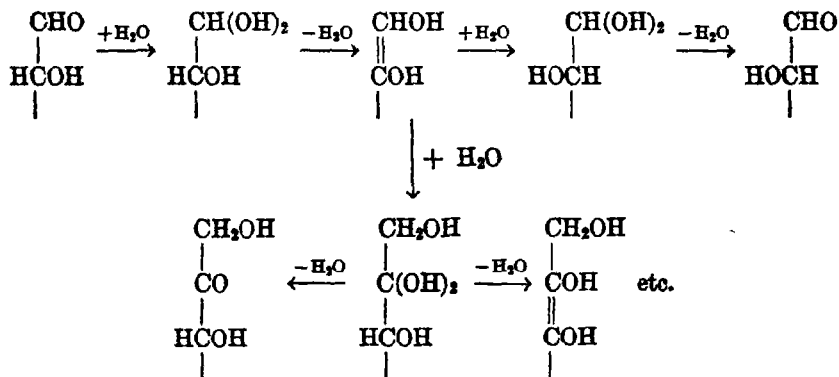
This mechanism accounts adequately for the known formation of glucose, fructose, and mannose from any one of the three used as starting material, as well as for the supposed presence of glutose (3-keto-hexose) and the kindred products which have been claimed as being formed. It may be considered the classical or basic theory of these changes. However, when Lewis and his co-workers[70] attempted to apply this same mechanism in the methylated sugar series they met with difficulty. Working under conditions which led only to the simplest changes, with little or no saccharinic acid formation, Lewis and Wolfrom studied the effect of alkali on tetramethylglucopyranose. True equilibrium was apparently established between the glucose and mannose

[69] Nef, *Ann.*, **335**, 191 (1904); **357**, 214 (1907); **376**, 1 (1910); **403**, 204 (1914).

[70] For the last publication of this series and for earlier references see Loder with Lewis, *J. Am. Chem. Soc.*, **54**, 1040 (1932).

derivatives, but they observed no ketose formation and on this basis argued that the views of Nef and Lobry de Bruyn should be replaced by the simpler concept of enolization. Thus:

$$
\begin{array}{ccc}
\text{CHO} & \text{CHOH} & \\
| & || & \\
\text{HCOH} & \text{COH} & \\
| & | &
\end{array}
\quad \text{or} \quad
\begin{array}{c}
\text{CH}_2\text{OH} \\
| \\
\text{CO} \\
| \\
\\
\text{CHO} \\
| \\
\text{HOCH} \\
|
\end{array}
$$
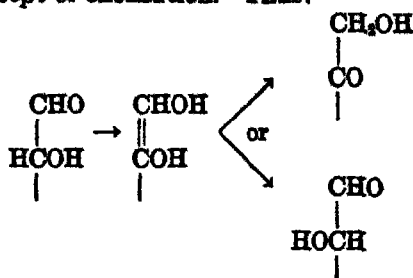
The changes predicted for the free sugars would be identical on either basis, but in the case of a methylated sugar Lewis considers that further enolization is blocked by the methyl group, owing to its non-mobility. It is his view that, on the basis of the Nef theory, water would be added, forming a hemiacetal which would readily lose methyl alcohol, and that subsequent changes would be similar in character.

$$
\begin{array}{ccc}
\text{CH(OH)}_2 & \text{CHOH} & \text{CH}_2\text{OH} \quad\quad \text{CH}_2\text{OH} \\
| & || & | \;\diagup^{\text{OH}} \quad\quad | \\
\text{CHOMe} \xrightarrow{-\text{H}_2\text{O}} \text{COMe} \xrightarrow{+\text{H}_2\text{O}} \text{C} \quad\quad \rightarrow \text{CO} \quad + \text{MeOH} \\
| & | & | \;\diagdown_{\text{OMe}} \quad\quad |
\end{array}
$$
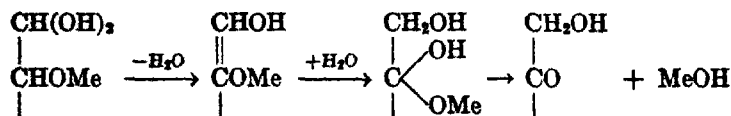
Against this it may be argued that this form of writing the reaction ignores the possibility of a lactol ring, and that if the ring form is considered then the postulated removal of the elements of water would give rise to tetramethyl-1,2-glucoseen:

$$
\overset{\displaystyle \overline{\text{O}}}{\text{CH}=\text{COMeCHOMeCHOMeCHCH}_2\text{OMe}}
$$
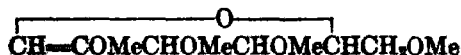
This compound has been prepared,[71] and a recent paper [71a] shows that it is stable to alkalies, and with acids gives rise to the methyl ether of hydroxymethylfurfural. These facts can be construed as additional evidence against the Nef mechanism.

In these studies on the methylated sugars it was observed that the apparent aldose content, as determined by hypoiodite titration, increased above 100 per cent but was reduced to normal on acidification. This was interpreted as proving that, in the methylated sugars, the assumed enediol has a tangible existence and that, by consuming more than one atom of oxygen per mole during oxidation, it is responsible for the high

[71] Wolfrom and Husted, *ibid.*, **59**, 2559 (1937).
[71a] Wolfrom, Wallace, and Metcalf, *ibid.*, **64**, 265 (1942).

analytical figures. Similar results were secured with the methylated pentoses, but here further complications arose, as methyl alcohol was split off and furfural was formed. It was found that the proportion of furfural increased with increasing "high iodine" value and also that the amount of methyl alcohol split off on acidification was about double that liberated in alkaline solution. This led to the formulation of a mechanism for the reaction as follows:

$$
\begin{array}{ccccc}
\text{CHO} & \text{CHOH} & \text{CHOH} & \text{CHO} \\
| & || & || & | \\
\text{CHOMe} & \text{COMe} & \lceil\text{—C} & \lceil\text{—C} \\
| & | & | & || \\
\text{CHOMe} \xrightarrow{\text{Alkali}} & \text{CHOMe} \xrightarrow{\text{Alkali}} & \text{O} | \text{ CHOMe} \xrightarrow{\text{Acid}} & \text{O} | \text{ CH} \\
| & | & | & | \\
\text{CHOMe} & \text{CHOMe} & | \text{ CHOMe} & | \text{ CH} \\
| & | & | & || \\
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \lfloor\text{—CH}_2 & \lfloor\text{—CH} \\
& & + & + \\
& & \text{MeOH} & \text{2MeOH}
\end{array}
$$
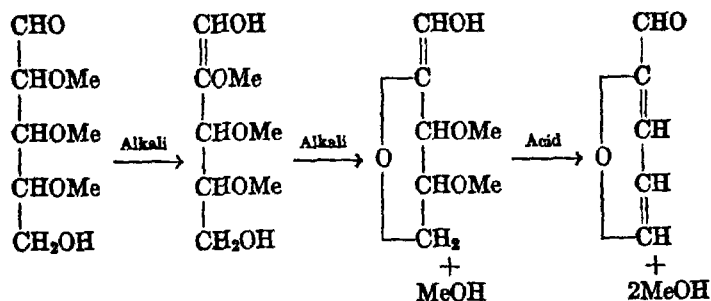
In this connection may be mentioned Hirst's observation that, when 2,3,4-trimethyl-δ-xylonolactone was heated with aqueous pyridine in the usual fashion in order to produce epimerization, the major product of the reaction was not the epimeric lyxonolactone but furancarboxylic acid. In this reaction no acid treatment is required to cause elimination of all methyl groups.

$$
\begin{array}{ccc}
\text{CO}\text{——}\rceil & \text{COOH} \\
| & | \\
\text{CHOMe} & \text{C}\text{——}\rceil \\
| & || \\
\text{CHOMe O} \rightarrow & \text{CH} \quad \text{O} \\
| & | \\
\text{CHOMe} & \text{CH} \\
| & || \\
\text{CH}_2\text{——}\rfloor & \text{CH}\text{——}\rfloor
\end{array}
$$
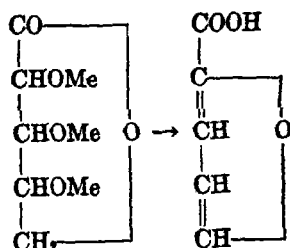
In all the investigations of Lewis the conditions, as mentioned above, were so chosen as to lead exclusively to the simplest changes. Evans and his collaborators, on the other hand, have done a vast amount of work under more drastic conditions, leading to more deep-seated changes in the molecule.[72] In these studies, glucose, fructose, and mannose were found to react analogously, and the products were formed in roughly the same amounts in each instance. The experiments were therefore interpreted on the assumption that the first product is the common enol,

[72] For most recent publication and earlier references see Plunkett and Evans, ibid., 60, 2847 (1938).

which then either undergoes scission or is attended by migration of the double bond farther down the chain. Thus:

$$
\begin{array}{cccccc}
\text{CHO} & \text{CHOH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} \\
| & \| & | & | & | & | \\
\text{CHOH} & \text{COH} & \text{CO} & \text{COH} & \text{CHOH} & \text{CHOH} \\
| & | & | & \| & | & | \\
\text{CHOH} & \text{CHOH} & \text{CHOH} & \text{COH} & \text{CO} & \text{COH} \\
| \rightarrow & | \rightarrow & | \rightarrow & | \rightarrow & | \rightarrow & \| \\
\text{CHOH} & \text{CHOH} & \text{CHOH} & \text{CHOH} & \text{CHOH} & \text{COH} \\
| & | & | & | & | & | \\
\text{CHOH} & \text{CHOH} & \text{CHOH} & \text{CHOH} & \text{CHOH} & \text{CHOH} \\
| & | & | & | & | & | \\
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH}
\end{array}
$$

Scission of each of these enediols was also assumed, with subsequent rearrangement of the fragments.

$$
\begin{array}{ccc}
\text{R} & \text{R} & \text{R} \\
| & | & | \\
\text{COH} & >\text{COH} & \text{CHO} \\
\| \rightarrow & \rightarrow & \\
\text{COH} & >\text{COH} & \text{CHO} \\
| & | & | \\
\text{R}' & \text{R}' & \text{R}'
\end{array}
$$

The intermediates above are written in the Nef methylenic form to suggest their reactive nature, for although rearrangements such as

$$\text{CH}_2\text{OHCOH}< \ \rightarrow \ \text{CH}_2\text{COOH}$$

were assumed, experimentally no acetic acid was formed from glycolic aldehyde under similar conditions. Glyceraldehyde and dihydroxy-acetone, on the other hand, did actually give rise to lactic acid as well as to pyruvic aldehyde (isolated as the osazone) although the yields were far from quantitative. These last-named substances may be included in the above scheme as follows:

$$
\begin{array}{ccccc}
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_3 & \text{CH}_3 \\
| & | & | & | & | \\
\text{CHOH} & \text{CHOH} & \text{CHOH} & \text{CO} & \text{CHOH} \\
| \rightarrow & | \rightarrow & | \rightarrow & | \rightarrow & | \\
\text{COH} & >\text{COH} & \text{CHO} & \text{CHO} & \text{COOH} \\
\| & & & & \\
\text{COH} & & & & \\
|
\end{array}
$$

as, quite obviously, may a great number of isomeric pentoses and tetroses.

In addition to the studies outlined above, Evans and his collaborators have extended the investigations to several other aldoses and

disaccharides and to hexosediphosphate. They considered that all the quantitative results were in accord with the general scheme outlined above and substantiated it in all important respects.

Before leaving the subject it seems well to point out certain less-emphasized phases of the problem. First of these is the question of conversion of aldehydic intermediates into corresponding acids, for example, the assumed production of acetic acid from acetaldehyde, or of formic acid from methylenenol ($=$CHOH). It is clear that both these changes involve an oxidation, which in turn demands intervention of atmospheric oxygen or else simultaneous reduction of some other product. In either event it would lead to the production of a large series of compounds which have been disregarded in the original scheme. It would seem necessary to be doubly cautious in considerations involving any products which do not have the empirical formula $(CHOH)_n \pm x H_2 O$. Actually Evans (private communication) has found that in a nitrogen atmosphere the peak of formic acid formation disappears, and he inclines to the view that even more rigorous exclusion of traces of oxygen is necessary.

A second assumption which appears to have been accepted in most studies of this sort is that of the reversible nature of the various reactions. In dealing with compounds of this type in which the free-energy differences are small, it is frequently possible to produce, at will, either forward or reverse reactions depending upon concentrations and conditions. This, in turn, has been taken as indication of true reversible reaction (in the physicochemical sense), and this has been in fact frequently assumed, either explicitly or implicitly.[73] It is evident that thermodynamic equilibrium demands an identical final composition of the mixture, no matter which component is used as starting material, yet in the many experiments which have been performed this seems not to be the tendency. In general the initial component predominates while the other products appear to form in almost random ratio. The extenuating circumstance in most of these experiments is that saccharinic acids frequently form and thus decrease the alkalinity. Also, as Evans has pointed out, other acids may be formed and thus disturb the equilibria. The need for many additional data on the subject of true reversibility is evident.

The final and perhaps the most important assumption which needs scrutiny is that concerning scission of the enol forms.[74] That ethylenic linkages are reasonably susceptible of rupture by oxidants appears to be adequately established, but simple hydrolytic scission, such as that

[73] Nef, Ann., 403, 206 (1914); Kusin, Ber., 69, 1041 (1936).
[74] Evans et al., J. Org. Chem., 1, 1 (1936); Schmidt, Ber., 68, 60 (1935); Neuberg, Ber., 68, 505 (1935).

assumed in the mechanisms above, is on a much less secure experimental basis. It would appear desirable to have more extensive data on simple non-oxidative cleavage of enediols before accepting, without reservation, mechanisms based on this type of reaction.

Saccharinic Acid Formation. It has been shown above that the simplest effect of alkali on a sugar is the catalysis of mutarotation, the next is enol formation and epimerization, while more deep-seated changes are those of migration of the double bond and cleavage into smaller fragments. Accompanying these last reactions is still another, that of intramolecular oxidation and reduction (or rearrangement), leading to the formation of the so-called saccharinic acids. These are respectively:

(a) Metasaccharinic acids   (eight possible
     $CH_2OHCHOHCHOHCH_2CHOHCOOH$ hexonic)

(b) Isosaccharinic acids   (four possible
                     $COOH$ hexonic)
    $CH_2OHCHOHCH_2COH \big\langle$
                     CH_2OH

(c) Saccharinic acids   (eight possible
                     $COOH$ hexonic)
    $CH_2OHCHOHCHOHCOH \big\langle$
                     CH_3

(d) Parasaccharinic acids   (four possible
                     $COOH$ hexonic)
    $CH_2OHCHOHCOH \big\langle$
                   CH_2CH_2OH

The formula in each case is $C_6H_{12}O_6$, so that each of these acids is isomeric with the parent hexose. Certain of the corresponding saccharinic acids from pentoses have been described by Nef, but these are analogous in character. Of the twenty-four possible isomers listed above, only a few have been described:

(a) One or two metasaccharinic acids from galactose or lactose and two from glucose.

(b) One or two isosaccharinic acids from maltose, lactose, or cellobiose but none from glucose or galactose.

(c) One saccharinic acid from glucose or mannose.

(d) One parasaccharinic acid from galactose or lactose.

The mechanism of the formation of these substances has been the subject of extensive research but is still incompletely understood. The earliest attempt to account for their formation was that of Kiliani, who assumed that lactic acid and glyceraldehyde, formed from the sugar under the influence of alkali, were recondensed to give the saccharinic acids. By assuming condensation of other acids and aldehydes this

mechanism was extended by Windaus to include all the isomeric saccharinic acids, but the theory has received little experimental support.

The most extensive investigations in the field were made by Nef, with his co-workers, in a series of classical researches. On the basis of the earlier work of Lobry de Bruyn, a progression of the carbonyl group down the carbon chain was postulated and the various ketoses thus formed were then assumed to undergo internal oxidation and reduction leading to the formation of desoxy diketo compounds. A benzilic acid rearrangement (p. 974) of these substances gave rise to the various saccharinic acids. Thus, as written by Nef, who assumed the reactive-methylenic intermediates:

$$
\begin{array}{c}
| \\
CO \\
| \\
CHOH \\
| \\
CHOH \\
|
\end{array}
\xrightarrow{-H_2O}
\begin{array}{c}
| \\
CO \\
| \\
C< \\
| \\
CHOH \\
|
\end{array}
\rightarrow
\begin{array}{c}
| \\
CO \\
| \\
CH \\
\diagdown O \\
CH \diagup \\
|
\end{array}
\rightarrow
\begin{array}{c}
| \\
CO \\
| \\
CO \\
| \\
CH_2 \\
|
\end{array}
\xrightarrow[\text{rearrangement}]{\text{Benzilic acid}}
\begin{array}{c}
| \\
C \diagup COOH \\
\diagdown OH \\
| \\
CH_2 \\
|
\end{array}
$$
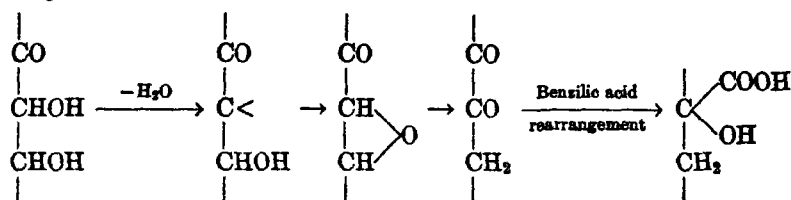
If this same scheme is followed, starting with the aldehydo-, 2-keto-, and 3-ketohexoses, it will be found that the first gives rise to the meta-saccharinic acids, the second to the isosaccharinic, and the third, where two diketo compounds are possible,

$$CH_2OHCHOHCOCHOHCHOHCH_2OH \Bigg\langle \begin{array}{l} {}^\nearrow CH_2COCOCHOHCHOHCH_2OH \\ {}_\searrow CH_2OHCHOHCOCOCH_2CH_2OH \end{array}$$
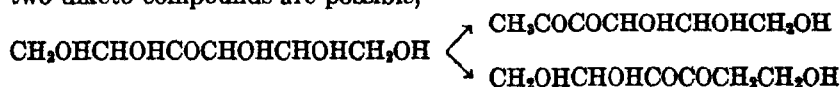
to both saccharinic and parasaccharinic acids. In the above scheme the formation of the diketo compounds might equally well be based on a selective removal of the elements of water followed by ketonization, as proposed by Lewis:

$$
\begin{array}{c}
| \\
CO \\
| \\
CHOH \\
| \\
CHOH \\
|
\end{array}
\xrightarrow{-H_2O}
\begin{array}{c}
| \\
CO \\
| \\
COH \\
\parallel \\
CH \\
|
\end{array}
\rightarrow
\begin{array}{c}
| \\
CO \\
| \\
CO \\
| \\
CH_2 \\
|
\end{array}
$$
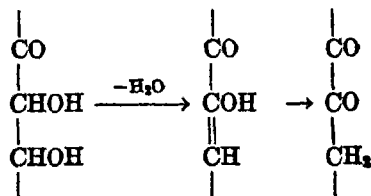
In recent years, Benoy and Evans have proposed a modification of Nef's scheme, based on their emphasis of the enediols as intermediate in these mechanisms.[75] The same enediols which they postulated to account for the isomerizations produced by alkali serve as intermediates in the saccharinic acid formation, and the one mechanism accounts for both types of reactions. These authors suggested an isomerization of the enediol as follows:

[75] Benoy and Evans, see *J. Am. Chem. Soc.*, **48**, 2676 (1926).

$$
\begin{array}{c}
| \\
COH \\
\parallel \\
COH \\
| \\
CHOH \\
|
\end{array}
\quad \xrightarrow{-H_2O} \quad
\begin{array}{c}
| \\
C \\
\parallel \!\! \diagdown \\
C \diagup \!\! O \\
| \\
CHOH \\
|
\end{array}
\quad \rightarrow \quad
\begin{array}{c}
| \\
CO \\
| \\
CO \\
| \\
CH_2 \\
|
\end{array}
$$
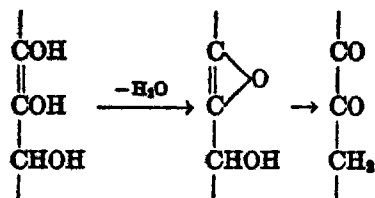
and the diketo derivative thus formed could undergo the benzilic acid rearrangement as postulated by Nef. The major difference between these two theories derives from the precursors which are assumed. as may be seen in the following scheme:

NEF   BENOY AND EVANS

Metasaccharinic ← aldehydo

                                      1,2-enediol → Saccharinic

Isosaccharinic
                        2-keto

                                      2,3-enediol ⟨ Parasaccharinic
                                                      Metasaccharinic

Saccharinic

Parasaccharinic ← 3-keto

                                      3,4-enediol → Isosaccharinic
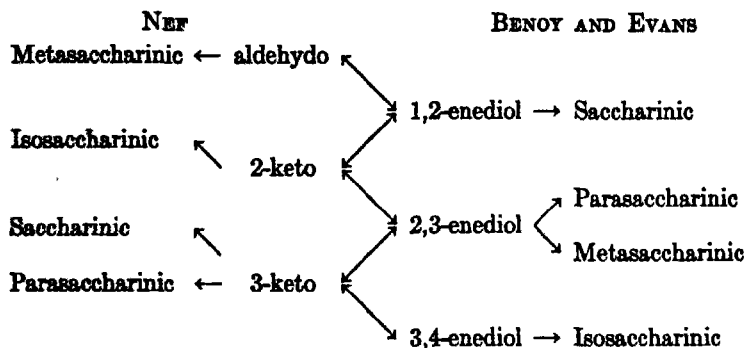
It would seem that quantitative studies on partially substituted hexoses or on the disaccharides might serve to decide between these two theories.

In recent years a further attempt to elucidate the mechanism of the formation of the saccharinic and isosaccharinic acids was made by Ohle. This author started from fructose, for example, and assuming a pinacol rearrangement, followed by selective removal and addition of water, formulated the desired substances.[75] Thus:

$$
\begin{array}{c}
CH_2OH \\
| \\
CO \\
| \\
CHOH \\
| \\
CHOH \\
| \\
CHOH \\
| \\
CH_2OH
\end{array}
\rightarrow
\begin{array}{c}
CH_2OH \\
| \\
COH \\
O\diagdown| \\
\diagup CH \\
| \\
CHOH \\
| \\
CHOH \\
| \\
CH_2OH
\end{array}
\rightarrow
\begin{array}{c}
CH_2OH\ COOH \\
\diagdown\diagup \\
CH \\
| \\
CHOH \\
| \\
CHOH \\
| \\
CH_2OH
\end{array}
$$

$$
\begin{array}{c}
CH_2\quad COOH \\
\diagdown\!\!\diagup \\
C \\
| \\
CHOH \\
| \\
CHOH \\
| \\
CH_2OH
\end{array}
\rightarrow
\begin{array}{c}
CH_2\quad COOH \\
\diagdown\diagup \\
COH \\
| \\
CHOH \\
| \\
CHOH \\
| \\
CH_2OH
\end{array}
$$

$$
\begin{array}{c}
CH_2OH\ COOH \\
\diagdown\diagup \\
C \\
\parallel \\
CH \\
| \\
CHOH \\
| \\
CH_2OH
\end{array}
\rightarrow
\begin{array}{c}
CH_2OH\ COOH \\
\diagdown\diagup \\
COH \\
| \\
CH_2 \\
| \\
CHOH \\
| \\
CH_2OH
\end{array}
$$
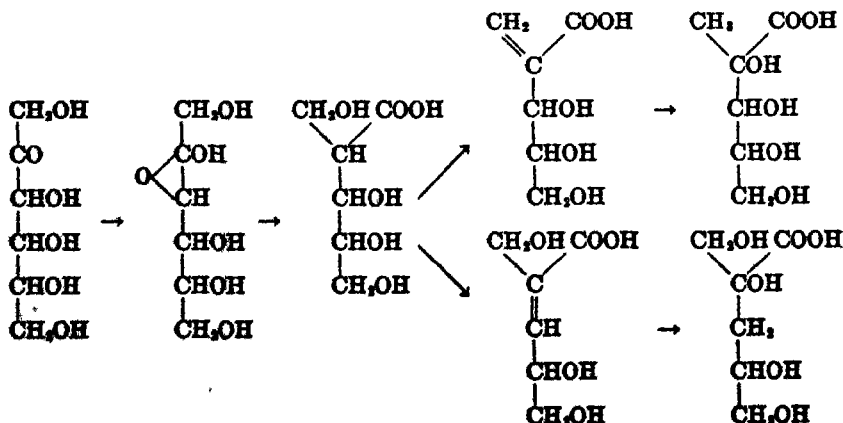
[75] Ohle, *Ergeb. Physiol.*, **33**, 606 (1931).

This mechanism cannot be used to account for the metasaccharinic and parasaccharinic acids.

There may also be mentioned the observation of Nicolet that α-hydroxy-β-methoxy-β-phenylpropiophenone, on treatment with alkali, gave α,β-diphenyllactic acid.[77] The author explained this on the basis of a benzilic acid transformation of the hypothetical diketone and suggested that the initial reaction (an "aldol dehydration") made a revision of Nef's theories necessary.

$$
\begin{array}{ccccc}
C_6H_5 & & C_6H_5 & C_6H_5 & C_6H_5 \\
| & & | & | & | \\
CHOCH_3 & & CH & CH_2 & CH_2 \\
| & -CH_3OH & \| & | & | \\
CHOH & \longrightarrow & COH \rightarrow & CO & \rightarrow HOCC_6H_5 \\
| & & | & | & | \\
CO & & CO & CO & COOH \\
| & & | & | & \\
C_6H_5 & & C_6H_5 & C_6H_5 &
\end{array}
$$
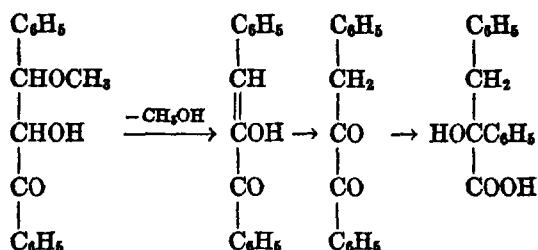
Evans (private communication), however, points out that, if the removal of methyl alcohol is assumed as the first step, the reaction may be represented as follows:

$$
\begin{array}{cccccc}
C_6H_5 & C_6H_5 & C_6H_5 & C_6H_5 & C_6H_5 & C_6H_5 \\
| & | & | & | & | & | \\
CHOCH_3 & CHOH & CHOH & CHOH & CH_2 & CH_2 \\
| & +H_2O & | & | & | & | & | \\
CHOH & \overrightarrow{-CH_3OH} & CHOH \rightarrow & COH & C & \rightarrow CO & \rightarrow HOCC_6H_5 \\
| & | & \| & & | & | \\
CO & CO & COH & -H_2O & CO & COOH \\
| & | & | & | & | \\
C_6H_5 & C_6H_5 & C_6H_5 & C_6H_5 & C_6H_5 &
\end{array}
$$
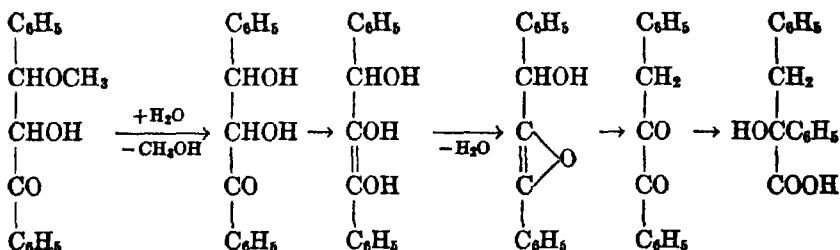
and thus included in the general mechanism outlined above.

Oxidation. In this discussion of oxidation, as in that of isomerization, it is convenient first to consider the reactions in *acid* medium since they are of less involved nature. In the presence of strong acids the results are complicated by the isomerizations which lead to the production of furfural derivatives, levulinic acid, and the humic acids. With bromine, on the other hand, such isomerizations are reduced to a minimum, and the reaction with aldoses is largely confined to simple oxidation to the corresponding acid:

$$Br_2 + H_2O + RCHO \rightarrow 2HBr + RCOOH$$
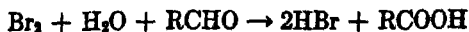
A similar conversion is achieved by using hot dilute nitric acid, and the major product is again the aldonic acid. This same reagent produces

[77] Nicolet, *ibid.*, **53**, 4458 (1931). See Nef, *Ann.*, **376**, 3 (1910).

selective oxidation of the primary alcoholic group adjacent to the reducing carbon in the case of ketoses, and has been found useful as a preparative method for the 2-keto aldonic acids which are intermediates in the ascorbic acid synthesis.

Boiling concentrated nitric acid oxidizes both terminal carbons and gives the dicarboxylic acids (saccharic acids), most often in the form of their mono- or dilactones. Thus:

$$
\begin{array}{cccc}
\text{CHO} & \text{COOH} & \text{CO}\!-\!\!\!-\!\!\!- \\
| & | & | \quad\quad\; | \\
\text{CHOH} & \text{CHOH} & \text{CHOH} \quad | \\
| & | & | \quad\quad\; | \\
\text{CHOH} & \text{CHOH} & \text{CHOH} \quad | \;\; \text{O} \\
| \quad\rightarrow & | \quad\rightarrow & | \quad\quad\; | \\
\text{CHOH} & \text{CHOH} & \text{CH}\!-\!\!\!-\!\!\!- \\
| & | & | \\
\text{CHOH} & \text{CHOH} & \text{CHOH} \\
| & | & | \\
\text{CH}_2\text{OH} & \text{COOH} & \text{COOH}
\end{array}
$$
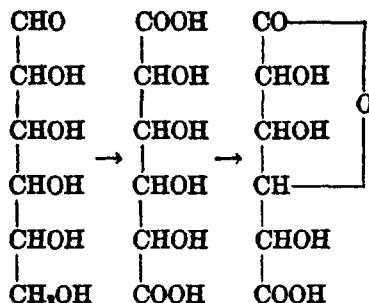
This same reagent is frequently used in structural determinations involving the methylated sugars, as they are attacked at the point of the lactol or lactone ring, the position of which is indicated by the nature of the oxidation products. For example,

$$
\begin{array}{ccccc}
\text{CHOMe}\!-\!\!- & \text{CO}\!-\!\!- & \text{COOH} & \text{COOH} & \text{COOH} \\
| \quad\quad | & | \quad\quad | & | & | & | \\
\text{CHOMe} \; | & \text{CHOMe} \; | & \text{CHOMe} & \text{CHOMe} & \text{CHOMe} \\
| \quad\quad | & | \quad\quad | & | & | & | \\
\text{CHOMe} \; \text{O} & \text{CHOMe} \; \text{O} & \text{CHOMe} & \text{CHOMe} & \text{CHOMe} \\
| \quad\quad | \;\rightarrow & | \quad\quad | \;\rightarrow & | & | & | \;\rightarrow \\
\text{CHOMe} \; | & \text{CHOMe} \; | & \text{CHOMe} & \text{CHOH} & \text{COOH} \\
| \quad\quad | & | \quad\quad | & | & | & \\
\text{CH}\!-\!\!- & \text{CH}\!-\!\!- & \text{COOH} & \text{CHOMe} & \\
| & | & & | & \\
\text{CH}_2\text{OMe} & \text{CH}_2\text{OMe} & & \text{CH}_2\text{OMe} &
\end{array}
$$
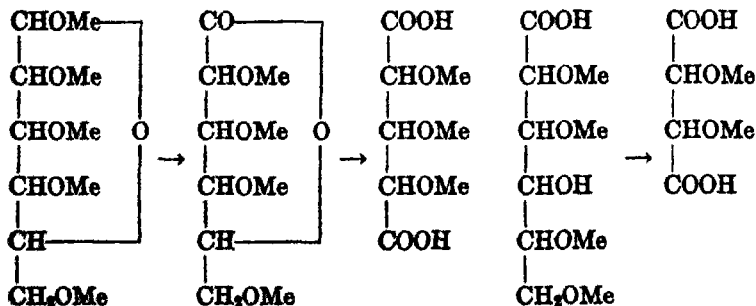
The method is similarly employed to determine the position of substituents, by fully methylating the substance, removing the substituent, oxidizing, and determining the nature of the oxidation products.

An oxidation which has preparative significance is that with hydrogen peroxide in the presence of iron catalysts. With ferrous salts both aldoses and 2-ketoses are converted to the osones, while fragments of the molecule appear as by-products in the form of the acids:

$$\text{RCHOHCHO} \rightarrow \text{RCOCHO} \leftarrow \text{RCOCH}_2\text{OH}$$

With ferric iron, notably colloidal ferric hydroxide, as catalyst the method becomes a useful one for the preparation of the aldoses of one less carbon from the aldonic acids:

$$RCHOHCOOH + (O) \rightarrow RCHO + CO_2 + H_2O$$

A similar oxidation has been achieved electrolytically, and a further special case of the same general reaction is the action of hypochlorites or hypobromites on the sugar acid amides:

$$RCHOHCONH_2 + OH^- + OCl^- \rightarrow RCHO + NCO^- + Cl^- + 2H_2O$$
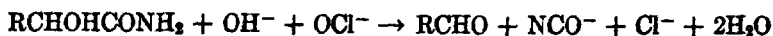
To Weerman is due the observation that this reaction may be used to prove substitution on the second carbon atom of aldoses, for formation of isocyanates does not then take place.[78]

In *alkaline* oxidations the various isomerizations which have already been discussed must also be taken into consideration. With weakly alkaline reagents, and at low temperatures, isomerization may be negligible, but with the more alkaline oxidants, particularly when used hot, it may predominate. An example of the first class is oxidation with hypoiodites, usually at room temperature or below. Simple oxidation of aldoses is observed, and under controlled conditions of alkalinity and temperature, ketohexoses are negligibly affected and the method is not only specific, but quantitative as well.

$$RCHO + I_2 + 2OH^- \rightarrow RCOOH + H_2O + 2I^-$$
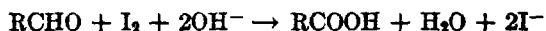
An oxidation, similar to this, has been achieved by Isbell, who performed an electrolytic oxidation in the presence of small amounts of bromides. Here the bromide may be considered as being continuously oxidized to the hypobromite, and this in turn as being continuously reduced by the aldose with the formation of the aldonic acid. A by-product of this reaction with glucose is 5-ketogluconic acid.[79] Oxidation of glucose directly with barium hypobromite leads to the production of a considerable amount of this 5-ketogluconic acid.[80] Recent work on the preparation and isolation of these keto acids has been done by Everett.[81]

Of theoretical importance in connection with the hypobromite oxidation is the conclusion of Isbell, who has presented evidence showing that there is a difference between the α- and β-forms of the aldoses as

[78] Weerman, *Rec. trav. chim.*, **37**, 16 (1917). See Ault, Haworth, and Hirst, *J. Chem. Soc.*, 1722 (1934).

[79] Cook and Major, *J. Am. Chem. Soc.*, **57**, 773 (1935).

[80] Reichstein and Nerscher, *Helv. Chim. Acta*, **18**, 892 (1935).

[81] Hart and Everett, *J. Am. Chem. Soc.*, **61**, 1822 (1939); Crews, Hart, and Everett, *ibid.*, **62**, 491 (1940).

regards the rate of their oxidation.[52] The same author also believes that the sugars are directly oxidized by this reagent from their lactol to their lactone forms, the ring being unchanged in the process.[53]

$$CH_2OHCHCHOHCHOHCHOHCHOH \xrightarrow{NaOBr} CH_2OHCHCHOHCHOHCHOHCO$$

Another reaction of hypobromites is the more vigorous treatment of ketoses which cleaves the molecule at the carbonyl group. The same result is secured by using mercuric oxide in alkaline solution:

$$RCHOHCOCH_2OH \xrightarrow{NaOBr} RCOOH + HOOCCH_2OH$$
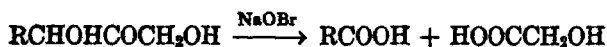
In recent years there has been described a most important reaction which involves the treatment of glycosides with hypobromites and leads to the complete elimination of the third carbon from the molecule, while the remainder is obtained as a mixed acetal: [54]

$$
\begin{array}{ccc}
CH_3OCH\!- & & CH_3OCH\!- \\
| & & | \\
CHOH & & COOH \\
| & & | \\
CHOH & & \\
| & O \to & O \\
CHOH & & COOH \\
| & & | \\
CH\!- & & CH\!- \\
| & & | \\
CH_2OH & & CH_2OH
\end{array}
$$
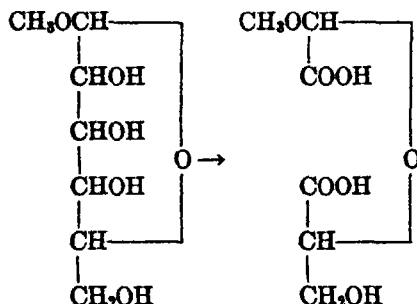
This reaction is discussed in the preceding chapter (p. 1569) in connection with structural determinations.

Many of the quantitative analytical reagents used for sugar determinations are more or less alkaline in reaction and, being used hot, produce fairly extensive isomerization. Sobotka has shown that, in the series of methylated sugars, oxidation by a typical sugar reagent diminishes progressively as the methyl groups are moved toward the reducing carbon.[55] Thus 3-methylglucose is much less reducing than glucose itself, and 2,3-dimethylglucose has very little reducing action. This observation has been confirmed with several of the monomethylglucoses and has been explained on the basis of isomerizations leading to the formation of non-reducing products such as the saccharinic acids. However, it is to be noted that, even in the case of free glucose, in the

[52] Isbell, J. Am. Chem. Soc., 54, 1692 (1932).
[53] Isbell and Hudson, J. Research Natl. Bur. Standards, 8, 327 (1932); Isbell, ibid., p. 615.
[54] Jackson and Hudson, J. Am. Chem. Soc., 58, 378 (1936).
[55] Sobotka, J. Biol. Chem., 69, 267 (1926).

normal oxidation time (by which time the reaction is approaching a maximum) only three atoms of oxygen have been consumed. This is equivalent to the production of the acid of one less carbon:

$$RCHOHCHO + 3(O) \rightarrow RCOOH + H_2O + CO_2$$
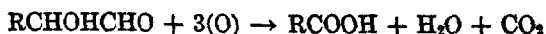
so that it is surprising that 3-methylglucose should be so much less reducing. Although this is certainly in part due to the isomerizations mentioned above, it appears possible that it may also be due to the prevention of mid-chain oxidations (like that with hypobromite) by the presence of the stable substituted groups.

This oxidation by alkaline reagents has been extensively studied by Nef [69] and more recently by Evans,[72] and these authors interpret the results on the basis of the intermediates produced by the action of alkali, and their subsequent reaction with the oxidant. Evans' emphasis is upon the enediols as intermediates, and it was his conclusion that the quantitative results were in satisfactory agreement with the assumption of cleavage and oxidation of the various enediol forms.

In the discussion above there have been considered only a few of the many reagents and reactions which have been studied, and of which there are an enormous number. Catalytic oxidation, oxidation by air, by peroxides, and by salts and oxides of numerous metals have been extensively studied. They are omitted here for the reason that in general they are similarly explained and usually permit qualitative if not quantitative interpretation on the assumption of enediols and their reactions. Two reagents which may be mentioned are the peracids and lead tetraacetate which are used for the oxidation of substances having double bonds in the molecule. As mentioned above, this last reagent has been found of theoretical and preparative use since it produces oxidation of the saturated sugars only if there are two adjacent unsubstituted hydroxyls. One final reagent which has found some application in structural determinations is silver oxide in aqueous solution.[86] There are indications that in the methylated sugars this reagent may produce oxidation only at the point of attachment of the lactol ring, although this has not as yet been firmly established.

As a final consideration in this section may be mentioned the work of Ohle and his collaborators,[87] directed towards securing "models" for the biological breakdown of carbohydrates. Working with fructose derivatives, they found as intermediates branched-chain dicarboxylic acids which they called furtonic acids. Thus β-diacetonefructose-1-

[86] See, for example, Freudenberg, *Ber.*, **59**, 836 (1926); Micheel, *Ber.*, **63** 347 (1930); Levene and Compton, *J. Biol. Chem.*, **112**, 775 (1936).

[87] Ohle, Contaicos, and Gonzalez, *Ber.*, **64**, 1759, 2804, 2810 (1931).

sulfate, on oxidation with permanganate, gave β-monoacetone-*l*-furtondi-carboxylic acid 1-sulfate, while β-diacetonefructose-1-carboxylic acid gave β-monoacetone-*l*-furtontricarboxylic acid. The last, on acid hydrolysis, yielded glycolic aldehyde, glycolic acid, and two molecules of carbon dioxide:

$$
\begin{array}{c}
CH_2OSO_3H \\
| \\
Me_2C{<}\begin{array}{c}OC{-}\\OCH\end{array} \\
| \\
CHO\!\!\diagdown\\
CMe_2 \\
CHO\!\!\diagup \\
| \\
CH_2{-}
\end{array}
\quad O \quad \rightarrow \quad
\begin{array}{c}
CH_2OSO_3H \\
| \\
Me_2C{<}\begin{array}{c}OCOH\\OCH\end{array} \\
| \\
CH \\
\diagup\;\diagdown \\
COOH\;\;COOH
\end{array}
$$

$$
\begin{array}{c}
COOH \\
| \\
Me_2C{<}\begin{array}{c}OC{-}\\OCH\end{array} \\
| \\
CHO\!\!\diagdown\\
CMe_2 \\
CHO\!\!\diagup \\
| \\
CH_2{-}
\end{array}
\quad O \quad \rightarrow \quad
\begin{array}{c}
COOH \\
| \\
Me_2C{<}\begin{array}{c}OCOH\\OCH\end{array} \\
| \\
CH \\
\diagup\;\diagdown \\
COOH\;\;COOH
\end{array}
\quad \rightarrow \quad
\begin{array}{c}
CH_2OHCOOH \\
\text{Glycolic acid} \\
+ \\
CH_2OHCHO \\
\text{Glycolic aldehyde} \\
+ \\
2CO_2
\end{array}
$$
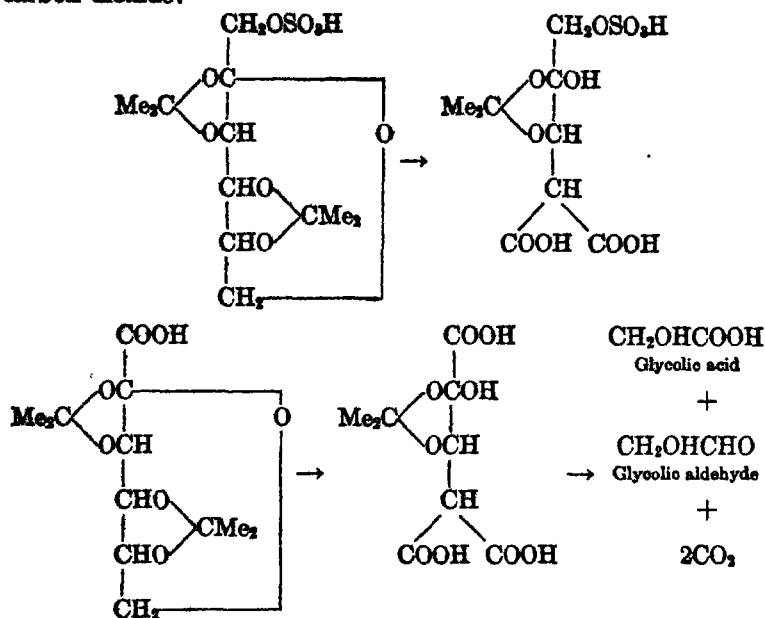
Similar reactions were observed with α-diacetonefructose-3-sulfate and with the corresponding phosphoric esters. On the other hand, it was found that monoacetoneglucose-3-sulfate on oxidation gave the 3-sulfate of monoacetonexyluronic acid and not a furtonic acid.

In spite of the very interesting nature of these reactions, considerable additional information will be needed before the results can be applied in any biological connections.

Fermentations

Alcoholic. One of the most important biological processes, and one of the most extensively studied, is the metabolism of carbohydrates. The best understood of these reactions is alcoholic fermentation, and, inasmuch as it may be used as a starting point for the discussion of almost all kindred processes, it may be considered at some length.

Live-yeast fermentation is confined to certain disaccharides, one of the two nonoses, a few hexoses, α-glucosan, 5-ketofructose, and the trioses, and on this basis it has been stated that only such sugars ferment as contain three or a multiple of three carbon atoms. In general it

appears that the disaccharides undergo preliminary hydrolysis to the hexoses, although some evidence exists to show that this is not invariably true and that they may be fermented directly. The trioses, moreover, are fermented only slowly and may perhaps undergo a preliminary conversion, the exact nature of which will be discussed later. In any event the significant fermentation is that of the hexoses, and of these only d-glucose, d-mannose, d-fructose, and (by specially cultured yeasts) d-galactose are utilized. Introduction of any substituent whatsoever * has been found invariably to prevent fermentation.

In live-cell fermentation it was found that a quantitative "balance-sheet" could be prepared in which, with fair precision, the fermentation is described by the equation:

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH \qquad (1)\dagger$$

However, working with the fermentation enzyme, Harden,[88] in his brilliant researches, made the astonishing discovery that inorganic phosphates are involved in the enzymic reaction which is more nearly represented by the equation:

$$2C_6H_{12}O_6 + 2H_3PO_4 \rightarrow 2CO_2 + 2C_2H_5OH + C_6H_{10}O_4(PO_4H_2)_2 + 2H_2O \quad (2)$$

Harden was able to isolate a large proportion of the organic phosphate required by this equation, and to show that the dynamics of the reaction was in essential accord with this mechanism. The fact that the simpler equation (1) applied in the live-cell fermentation was explained by assuming that, in the intact cell, a mechanism exists for the rapid hydrolysis of the hexosediphosphate, thus regenerating inorganic phosphate and fermentable hexose:

$$C_6H_{10}O_4(PO_4H_2)_2 + 2H_2O \rightarrow C_6H_{12}O_6 + 2H_3PO_4 \qquad (3)$$

The net result of (2) and (3) is (1).

Such hydrolysis does, in fact, occur in the enzymic system but at so low a rate that the diphosphate accumulates during rapid fermentation. It is of interest in this connection that the addition of arsenates or arsenites increases the rate of enzymic fermentation, which, in special cases, may approach that of the living yeast equivalent to the amount of

* Exceptions to this statement are the glycosides, but in general they are hydrolysed prior to fermentation.

† From data given in Parks and Huffman, "The Free Energies of Some Organic Compounds," Chemical Catalog Co., New York (1932), it may be calculated that for this reaction (CO_2 at 1 atm., C_2H_5OH and $C_6H_{12}O_6$ in 1 molal solution)

$$\frac{(C_2H_5OH)^2[CO_2]^2}{(C_6H_{12}O_6)} = 10^{10} \quad (\text{approx.})$$

In view of this fact the irreversibility of fermentation in actual practice is not surprising.

[88] See Monograph, "Alcoholic Fermentation," by Harden [Longmans, Green and Co., London (1932)].

enzyme used. This has been explained by assuming that arsenates increase the rate of reaction (3), regenerating inorganic phosphate more rapidly and thus accelerating reaction (2).

Another significant fact, which must receive consideration, is derived from experiments on certain enzyme preparations which show a delayed starting or induction period, followed by normal fermentation. It has been found that certain of the phospho esters, but especially the hexosediphosphate, in very low concentration, reduce or entirely abolish this induction period so that fermentation starts almost immediately. Nothing in the preceding reactions permits prediction of this surprising result, which seems to indicate the diphosphate to be some sort of intermediate in the fermentation mechanism. Against this view is the fact that the diphosphate exhibits a very low rate of enzymic fermentation, whereas a true intermediate should ferment at least as fast as the parent hexose under equivalent conditions. To reconcile these two facts, recourse was usually had to the rather unsatisfactory explanation that the diphosphate was liberated in an "active" state which had a higher rate of fermentation than the "stable" forms which were isolated.

Within the past few years the problem has been entirely reopened and given a tremendous impetus by the important observations of Meyerhof and Lohmann, who found that an equilibrium is established with extraordinary speed between dihydroxyacetone phosphate and hexosediphosphate in the presence of yeast enzyme.[89]

$$C_6H_{10}O_4(PO_4H_2)_2 \rightleftarrows 2C_3H_5O_2(PO_4H_2)$$

Hexosediphosphate   Dihydroxyacetone phosphate
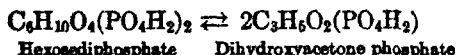
This constitutes one of the most significant observations thus far made in the field of carbohydrate metabolism, as here for the first time is a mechanism for securing the smaller triose units which have been so often postulated. Not only is considerable rearrangement involved in the reaction, but also an interchange of optically active and optically inactive material:

$$
\begin{array}{ccc}
CH_2OPO_3H_2 & & CH_2OPO_3H_2 \\
| & & | \\
CO & & CO \\
| & & | \\
HOCH & \rightleftarrows & CH_2OH \\
| & & | \\
HCOH & & CH_2OH \\
| & & | \\
HCOH & & CO \\
| & & | \\
CH_2OPO_3H_2 & & CH_2OPO_3H_2 \\
\end{array}
$$
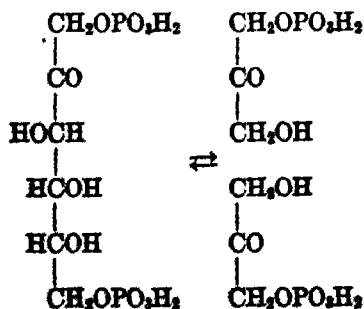
[89] Meyerhof and Lohmann, *Naturwissenschaften*, **22**, 134 (1934); *Biochem. Z.*, **271**, 89 (1934); **272**, 413 (1934); **275**, 430 (1935).

The change is reversible, and it is of great interest that true thermodynamic equilibrium is attained.

Following this major discovery, a new activity in this research field has brought to light a series of most remarkable reactions. The problem is currently in a transitional state, and constantly appearing new data make the subject difficult to present. However, it seems useful to present a complete theory and to indicate some of its deficiencies. This is perhaps best achieved by presenting the mechanism proposed by Meyerhof and Kiessling, which is as follows.[90]

$$C_6H_{12}O_6 + 2H_3PO_4 + C_6H_{10}O_4(PO_4H_2)_2 \rightleftarrows$$
Hexose, Hexosediphosphate

$$\left[4CH_2OPO_3H_2COCH_2OH \rightleftarrows 4CH_2OPO_3H_2CHOHCHO \right] \rightleftarrows$$
Phosphodihydroxyacetone, Phosphoglyceraldehyde

$$2CH_2OPO_3H_2CHOHCH_2OH + 2CH_2OPO_3H_2CHOHCOOH \quad (4)$$
a-Phosphoglycerol, 3-Phosphoglyceric acid

$$2CH_2OPO_3H_2CHOHCOOH \rightleftarrows 2CH_2OHCHOPO_3H_2COOH \quad \left.\right]$$
3-Phosphoglyceric acid, 2-Phosphoglyceric acid

$$2CH_2OHCHOPO_3H_2COOH \rightleftarrows 2CH_2{=}COPO_3H_2COOH + 2H_2O \quad \left.\right]^{(5)}$$
2-Phosphoglyceric acid, Phosphopyruvic acid enol

$$2CH_2{=}COPO_3H_2COOH + C_6H_{12}O_6 \rightarrow$$
Phosphopyruvic acid enol, Hexose

$$2CH_3COCOOH + C_6H_{10}O_4(PO_4H_2)_2 \quad (6)$$
Pyruvic acid, Hexosediphosphate

$$2CH_3COCOOH \rightarrow 2CH_3CHO + 2CO_2 \quad (7)$$
Pyruvic acid, Acetaldehyde

$$2CH_3CHO + C_6H_{12}O_6 + 2H_3PO_4 \rightleftarrows [\text{primary ester} + 2CH_3CHO]$$
Acetaldehyde, Hexose

$$\rightarrow 2CH_2OPO_3H_2CHOHCOOH + 2C_2H_5OH \quad (8)$$
3-Phosphoglyceric acid, Alcohol

The summation of 5, 6, 7, and 8 gives

$$2C_6H_{12}O_6 + 2H_3PO_4 \rightarrow C_6H_{10}O_4(PO_4H_2)_2 + 2CO_2 + 2C_2H_5OH + 2H_2O$$

identical with reaction (2) discovered by Harden. In order to make clearer the sequence of events leading to this result, the above mechanism is given in diagrammatic form below.

In this diagram there may be seen the utilization of glucose and inorganic phosphate and the production of hexosediphosphate, alcohol,

[90] Meyerhof and Kiessling, ibid., 281, 249 (1935); 283, 83 (1935).

See also the earlier basic results of Embden. For example, Embden, Deuticke, and Kraft, Klin. Wochschr., 12, 213 (1933); Embden and co-workers, Z. physiol. Chem., 230, 1 (1934).

and carbon dioxide, while the other products are formed and consumed in a cyclic manner.
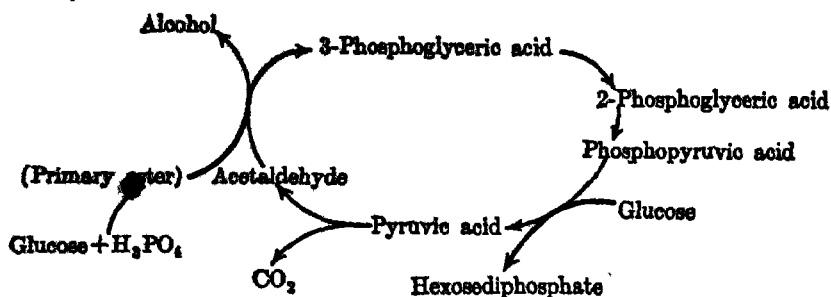
In the mechanism as outlined in the equations above, the only hypothetical substance is the primary ester which is assumed in reaction (8). With this exception all the substances indicated have been isolated, and many have been made available synthetically. The products have usually been demonstrated by employing incomplete systems (such as one lacking coenzyme) or partially impaired enzymes (such as those poisoned by iodoacetate or fluoride); under these circumstances the intermediates accumulate and can be isolated. Although there is some reason to discount conclusions based upon such abnormal enzyme systems,[21] still the method provides valuable data which must not be ignored.

The experimental basis for this mechanism is as follows:

Equation (4): Although hexosediphosphate alone is only slowly fermented by yeast juice, in the presence of glucose and inorganic phosphate it is very rapidly converted into equimolal quantities of α-phosphoglycerol and 3-phosphoglyceric acid. This presumably occurs through the intermediates, phosphodihydroxyacetone and phosphoglyceraldehyde, and there is evidence indicating the reversible interconversion of these two substances.

Equations (5): 3-Phosphoglyceric acid (which, incidentally, has also been prepared synthetically) undergoes a two-stage reaction, each step being reversible, whereby (levo)3-phosphoglyceric acid is converted into (dextro)2-phosphoglyceric acid. This in turn loses the elements of water and gives the phosphate of the enol form of pyruvic acid. This final product has also been prepared synthetically.

$$
\begin{array}{ccc}
CH_2OPO_3H_2 & CH_2OH & CH_2 \\
| & | & \| \\
CHOH & \rightleftarrows \ CHOPO_3H_2 & \rightleftarrows \ COPO_3H_2 \\
| & | & | \\
COOH & COOH & COOH \\
\text{(levo)} & \text{(dextro)} &
\end{array}
$$
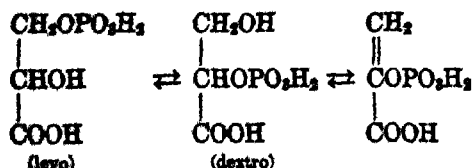
[21] See, for example, Nord, *Chem. Rev.*, **26**, 423 (1940).

Equation (6): If glucose is added to either natural or synthetic phosphopyruvic acid in the presence of the enzyme system, pyruvic acid and hexosediphosphate are formed by interchange of the phospho groups.

Equation (7): The pyruvic acid formed in reaction (6) is decarboxylated by the long-known enzyme *carboxylase* and yields acetaldehyde and carbon dioxide.

Equation (8): Acetaldehyde, glucose, and inorganic phosphate have been shown to react rapidly in the presence of hexosediphosphate and the yeast enzyme (through an assumed intermediate ester), reducing the aldehyde to alcohol and regenerating the original 3-phosphoglyceric acid. In this way the concentration of the latter is kept constant.

As will be seen from this discussion, the proposed mechanism rests on a fairly secure experimental basis. Moreover, it includes certain other phenomena connected with fermentation which cannot be discussed here. However, it cannot be stated with certainty that the above is the actual sequence of reactions in normal, unimpaired fermentation, and it is to be noted that certain of the proposed reactions [(8), for example] are of extremely high order. It seems probable that in such cases intermediate reactions occur which have not, as yet, been demonstrated, and that the actual mechanism will eventually be shown to embody many simultaneous but low-order reactions. Moreover, although hexosediphosphate is written in all the above equations, Meyerhof intends this to include monophosphates as well, and this relationship is still obscure. Finally it is to be noted that the "primary ester," which is postulated above, is considered to be an unstable phospho ester, not identical with any of those already known. The search for an intermediate of this nature dates back to Harden's original discovery of the participation of phosphates in the fermentation system.

In addition to the studies discussed above, mention should be made of the work of Schäffner and co-workers,[92] who prepared mixtures of purified enzymes obtained from various sources, and who by use of these "synthetic" fermentation systems were able to produce the following reactions:

$$CH_2OHCOCH_2OPO_3H_2 + CH_3CHO + H_2O$$

$$\rightarrow COOHCHOHCH_2OPO_3H_2 + C_2H_5OH \qquad (9)$$

and

$$C_6H_{12}O_6 + 2H_3PO_4 \rightarrow C_6H_{10}O_4(PO_4H_2)_2 + 2H_2O \qquad (10)$$
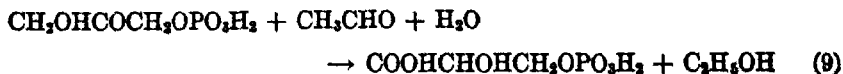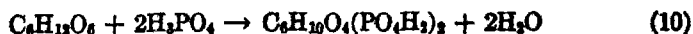
Reaction (9), by some obscure mechanism, induces reaction (10), and both are simultaneously inhibited by iodoacetic acid. These reactions,

[92] Schäffner and Bauer, *Naturwissenschaften*, **22**, 464 (1934); Schäffner, Bauer, and Berl, *Z. physiol. Chem.*, **232**, 213 (1935); **234**, 146 (1935).

though not of immediate relationship to the mechanism above, nevertheless appear to be of considerable significance.

In addition to the simple fermentation (1), the mechanism of Meyerhof and Kiessling may also be employed to explain some of the older observations of Neuberg.[88] In an attempt to "fix," or remove from the reaction, possible aldehydic intermediates this author added sulfites or alkali to both live-yeast and enzymic fermentations and in this way was able to produce two reactions:

$$C_6H_{12}O_6 \rightarrow C_3H_8O_3 + CH_3CHO + CO_2 \qquad (11)$$
$$\text{Glycerol}$$

$$2C_6H_{12}O_6 + H_2O \rightarrow 2C_3H_8O_3 + CH_3COOH + C_2H_6OH + 2CO_2 \qquad (12)$$
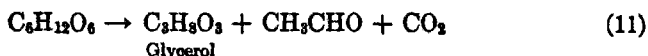
It will be noted that (12) would result by combining a "Cannizzaro" reaction, $2CH_3CHO + H_2O = CH_3COOH + C_2H_5OH$, with (11). The existence of an enzyme which accomplishes this type of change has in fact been demonstrated. Reaction (11) may be explained, on the basis of the Meyerhof-Kiessling mechanism, as resulting from removal of the acetaldehyde so that reaction (8) can no longer occur, and the 3-phosphoglyceric acid is therefore not regenerated. Thus, to replace it, (4) must proceed, and α-phosphoglycerol continues to be formed and to be converted to the glycerol indicated above.

Muscle Metabolism. Many of the data discussed above have been secured in studies on the closely related problem of muscle metabolism. This highly important mechanism has been included by Meyerhof and Kiessling in a similar scheme, in which reactions (4), (5), and (6) are the same and (7) and (8) are replaced by:

$$2CH_3COCOOH + 2H_3PO_4 + C_6H_{12}O_6$$

$$\rightarrow \text{primary ester} + 2CH_3COCOOH$$

$$\rightarrow 2 \text{ triose phosphate} + 2CH_3COCOOH$$

$$\rightarrow 2CH_2OPO_3H_2CHOHCOOH + 2CH_3CHOHCOOH \qquad (13)$$
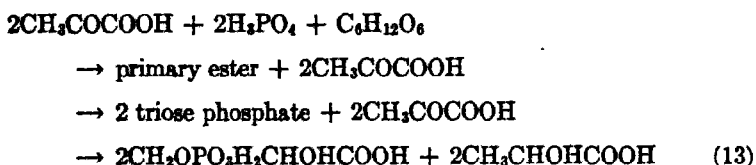
The phosphoglyceric acid for (5) is thus similarly regenerated, but the product of the reaction is lactic acid instead of alcohol and carbon dioxide.

An additional reaction which has been demonstrated in the muscle system is

$$C_6H_{12}O_6 + 2 \text{ adenylpyrophosphate} \rightarrow C_6H_{12}O_4(PO_4H_2)_2 + 2 \text{ adenylic acid} \qquad (14)$$

This is of considerable interest in connection with the problems of coenzyme and hexosediphosphate formation, but its implications cannot be considered here.

Other Fermentations. The mechanism just described can quite obviously be employed to describe the important lactic acid fermentation, for the net initial reaction is the same:

$$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH$$

although in the muscle the lactic acid then undergoes further transformation. That the above mechanism is indeed applicable to the lactic acid fermentation is partially substantiated by experimental evidence, but this is not yet nearly so complete as that for alcoholic fermentation.

In view of the tremendous amount of research which has been required to advance the understanding of these well-known processes to its present stage, it is not surprising that in the less-explored fields there should be little real knowledge. The ingenious and plausible mechanisms which might be written are largely without experimental verification and for some the enzyme systems have not even been isolated. Thus for the moment it seems desirable to postpone consideration of possible mechanisms until adequate data have been accumulated. Some of the commoner fermentations are indicated below, but in most of these reactions two or more processes appear actually to be taking place simultaneously, for the proportions of the various products may be changed by modifying the conditions. These are, therefore, to be considered as idealized equations which serve chiefly to indicate the nature of the products formed.

Butyric Acid Fermentation. A suggested mechanism is the following:

$$C_6H_{12}O_6 \rightarrow 2C_3H_6O_3 \rightarrow 2CH_3CHO + 2HCOOH$$

$$CH_3CHO + H_2O \rightarrow CH_3COOH + H_2 \text{ and } HCOOH \rightarrow CO_2 + H_2$$

$$2CH_3CHO \rightarrow CH_3CHOHCH_2CHO$$

$$\rightarrow CH_3CH{=}CHCHO + H_2O \rightarrow CH_3CH_2CH_2COOH$$
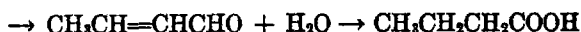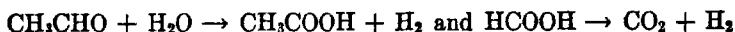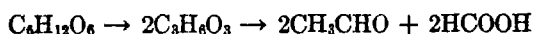
This mechanism accounts for the main products of the reaction, which are butyric and acetic acids, carbon dioxide, and hydrogen; a trace of formic acid is always present. The intermediate triose postulated above is presumably formed by the same sort of mechanism as in alcoholic fermentation and may actually be a phospho ester.

Butyl Alcohol and Acetone. The mechanism is like that above, with the addition of the following reactions:

$$2CH_3COOH \rightarrow CH_3COCH_2COOH \rightarrow CH_3COCH_3 + CO_2$$

$$CH_3COCH_3 + 2H \rightarrow CH_3CHOHCH_3$$

$$CH_3CHO + 2H \rightarrow CH_3CH_2OH$$

$$CH_3CH_2CH_2COOH + 4H \rightarrow CH_3CH_2CH_2CH_2OH + H_2O$$
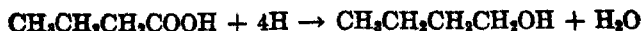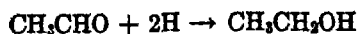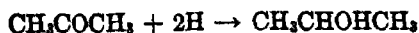
In this way, butyl alcohol, acetone, and ethyl and isopropyl alcohols are added to the products above. The first two are the dominant reaction products, and the fermentation has considerable commercial utility for production of these substances.

Propionic Acid. The intermediate here appears to be lactic acid:

$$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH$$

$$CH_3CHOHCOOH \rightarrow CH_3COCOOH + 2H$$

$$CH_3COCOOH \rightarrow CH_3CHO + CO_2$$

$$CH_3CHO + H_2O \rightarrow CH_3COOH + 2H$$

$$CH_3CHOHCOOH + 2H \rightarrow CH_3CH_2COOH + H_2O$$
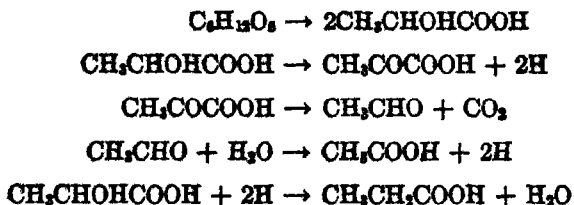
Propionic and acetic acids predominate, although other by-products are formed.

Citric Acid. The formation of a tribasic acid manifestly involves a very complex reaction, but the equation may be written in a simple form:

$$C_6H_{12}O_6 + H_2O \rightarrow C_6H_8O_7 + 6H$$
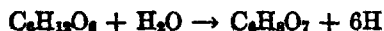
Acceptors for the hydrogen may be postulated in numerous ways.

Xylose Fermentation. Among the many pentose fermentations may be mentioned one in which xylose is fermented to the extent of 85–90 per cent to an equimolar mixture of acetic and lactic acids:

$$C_5H_{10}O_5 = CH_3COOH + CH_3CHOHCOOH$$

Oxidation by Acetobacter suboxydans. This organism produces dehydrogenations of the general type, $\overset{|}{\underset{|}{C}}HOH \rightarrow \overset{|}{\underset{|}{C}}O + 2H$. In this manner alcohol is converted to acetaldehyde, isopropyl alcohol to acetone, glycerol to dihydroxyacetone, sugar alcohols to ketoses, and gluconic acid to 5-ketogluconic acid. In the sugars the CHOH group adjacent to the primary alcohol group is converted to the ketose.

Oxidation by Acetobacter xylinum. The initial oxidations are identical with those above, but further oxidation of the products also occurs.

It is to be noted that in all these reactions the essential feature is the transfer of hydrogen by one or more mechanisms, and this may be considered as fundamental to all these biological processes. However, as in the alcoholic fermentation, the mechanism of this transport may prove to be highly complex.

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CHAPTER 22

CARBOHYDRATES III—CELLULOSE

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CONTENTS

INTRODUCTION

Viewed by the organic chemist, cellulose, the main constituent of all living plants, may be termed a natural high polymer the building unit of which is an anhydride of glucose. Nature, it is now believed, produces this polysaccharide in the form of long-chain molecules, possibly by condensation and dehydration of glucose.

However, cellulose as it exists in plants, as well as in its isolated state, possesses a complex physical structure which manifests itself in various forms; for example, as hair in cotton, as bast in flax, and as fiber in wood.* X-ray analysis, the primary means of investigating the fine structure of natural polymers, has definitely shown cellulose to be of crystalline nature. As a result, the individual chain molecules are thought to be arranged parallel to each other and to be stabilized laterally by secondary valences or by hydrogen bonds between opposing hydroxyl groups. The chain bundles thus formed are conceived to represent hypothetical submicroscopic units, of varying length but of fixed breadth and thickness, which are termed crystallites or micellae. The micellae build up the fibrillae, the first constituents of the fiber which may be detected under the microscope.

In defining the term cellulose, distinction should be made between cellulose as it exists in the plant and cellulose as it is obtained in its isolated form, for in the plant it may be chemically combined with other plant constituents, such as non-cellulosic carbohydrates or their acids and not merely mechanically associated with these and other compounds. On isolation and subsequent thorough purification cellulose may be obtained which is practically free of non-cellulosic substances. Thus, in isolated cellulose certain groups may be altered from the form which they possessed when still combined with groups of non-cellulosic substances. It is also possible that in cellulose, while it is still in the plant, opposing hydroxyl groups of the parallel chains, besides being associated through secondary valences or through hydrogen bridges, are in actual chemical combination at occasional points along the chains. Again, on isolation and purification, such cross linkages may undergo cleavage. Moreover, as yet no means are available which would permit isolating cellulose from its various sources without hydrolysis of a smaller or greater number of glycosidic linkages of the individual chains. As a result, isolated cellulose very likely possesses a shorter average chain length than cellulose in the plant. Finally, on isolation and purification, oxidation may take place and thus give rise to a further change of

* The term fiber is now used for all forms.

certain groups in the isolated product. Since almost all cellulose reactions have been and are undertaken with isolated cellulose, a discussion of these reactions and their evaluation will necessarily refer to cellulose as the isolated substance, unless otherwise stated.

In dealing with the chemistry of cellulose we cannot afford to neglect either its microscopic and submicroscopic morphological structure or, as a result of its high-polymeric character, its colloidal nature as manifested in its solid form as well as in dispersions and solutions. On the contrary, these peculiarities call for close consideration for they cause the reactions which cellulose undergoes to take a more or less heterogeneous course and usually to proceed at a relatively slow rate. On the other hand, its fibrous structure and its colloidal nature explain the enormous utility of cellulose for a great variety of purposes.

Cellulose may be obtained from any plant. The process of isolation, whether carried out in the laboratory or in commercial practice, is always the same in principle; that is, the raw materials are subjected to agents which are expected to dissolve or destroy the non-cellulosic substances but to have as little effect as possible upon the cellulose itself. The chief commercial raw materials are cotton, flax, and hemp for the textile industry, and the various species of wood for the pulp and paper industry. Cotton, in the form of cotton linters, also serves as raw material for the manufacture of cuprammonium and acetate rayon as well as for the preparation of most of the cellulose esters and ethers, but the viscose rayon industry uses wood pulp almost exclusively.

Whereas the small percentage of impurities is relatively easily removed from the cotton fiber, the isolation of cellulose from wood requires a far more drastic treatment. The separation of cellulose from lignin, the other main constituent of wood, may be accomplished by the treatment of wood either with a combination of calcium or sodium bisulfite and sulfurous acid (sulfite process) or with sodium hydroxide alone (soda process) or in combination with sodium sulfide (sulfate process). These treatments are carried out under pressure and at elevated temperature and are followed by processes of bleaching with chlorine and alkali hypochlorite. The wood pulp thus obtained may be further purified by extraction with sodium hydroxide solution whereby the percentages of non-cellulosic carbohydrates, such as pentosans (xylan), hexosans (mannan), and short-chain cellulose material (*beta-* and *gamma-*cellulose), are further reduced and the percentage of long-chain cellulose material (*alpha-*cellulose, characterized by its resistance to 17 to 18 per cent sodium hydroxide solution) is correspondingly increased.

The purest cellulose, which as "standard cellulose"* is used for experimental studies, may be obtained from raw cotton. After fat, wax, and other soluble impurities have been removed by extraction with organic solvents, the residue is freed of other non-cellulosic substances, such as pectin, by careful treatment with dilute alkali, and, after washing, is bleached slightly with hypochlorite or other bleaching agents.

Small amounts of cellulose occur in certain animal tissues (like *Phallusia mammillaris*), from which a type of animal cellulose, tunicin, may be obtained. Tunicin has been found to be identical with cotton cellulose.[1] As a material in which the chain molecules reveal particularly pronounced parallel orientation, it has often been used in comparative x-ray studies.[2]

The now generally accepted concept of the chemical constitution of the cellulose chain molecule, which is presented in Fig. 1, is the result of
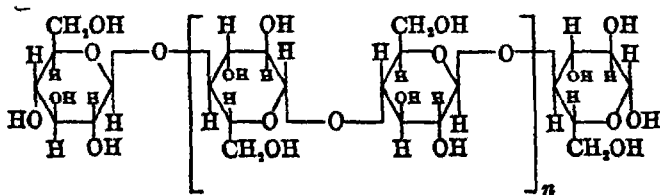
FIG. 1.

more than one hundred years of research on the behavior of cellulose with the most varied treatments. The reactions in question resemble those which are observed to occur with the simple sugars. Since, however, of the reducing groups of glucose, all but one (the one terminating unit of the open chain) are involved in the glycosidic linkings between individual glucose anhydrides, cellulose lacks the pronounced reducing power of most of the sugars, and its chief reactions are a result of its hydroxyl groups. As the formula shows, all glucose anhydrides except one (the other terminating unit) possess three free hydroxyl groups, one, in the 6-position, being of primary nature, and those in the 2- and in the 3-positions of secondary nature. The terminating unit which possesses four free hydroxyl groups has the additional hydroxyl group in the 4-position.

The free hydroxyl groups in cellulose react as in alcohols to form addition compounds with alkalies, and certain complex salts. Under

* This is the American Chemical Society method. See Corey and Gray, *Ind. Eng. Chem.*, **16**, 853, 1130 (1924). See, also, Schwalbe, *Papier-Fabr.*, **24**, 769 (1926); Werner and Macee, *J. Research Natl. Bur. Standards*, **21**, 609 (1938).

[1] Winterstein, *Ber.*, **26**, 362 (1893); Abderhalden and Zemplén, *Z. physiol. Chem.*, **72**, 58 (1911); Zechmeister and Tóth, *ibid.*, **215**, 267 (1933).

[2] Herzog and Gonell, *Z. physiol. Chem.*, **141**, 88 (1914).

special conditions, they also react with sodium metal to form compounds comparable with the simple alcoholates (alkoxides). Furthermore, the hydroxyl groups of cellulose react to form esters and ethers, and, on oxidation, are converted stepwise into aldehydic and carboxylic groups. In all these reactions cellulose behaves as an aliphatic polyalcohol.

Unless rigid precautions are taken, the introduction of new groups as well as oxidation are accompanied by hydrolytic attack of the glycosidic linkages which results in a shortening of the chain molecules. However, the chains obviously are long enough to endure frequent cleavage before they lose their polymeric and hence their cellulosic character.

The aggregate of chain molecules which comprises the cellulosic substance may not consist exclusively of glucose anhydride chains. Under certain conditions cellulose carefully isolated from the plant reveals the presence of carboxylic groups, which may be interpreted to mean that nature has oxidized the free reducing groups or even some or all of the primary alcoholic groups of some of the chains in the micelles. Oxidation of all the primary hydroxyl groups in a glucose anhydride chain would result in the formation of polyglucuronic acid. In a second phase nature might convert polyglucuronic acid into xylan by way of decarboxylation. Thus cellulose as it exists in the plant may contain some polyglucuronic acid and some xylan chains. The possible occurrence of such biochemical processes is suggested by the fact that both polyuronic acids and xylan are found to be associated with cellulose in many plants. It might also explain the difficulty of freeing cellulose, prepared, for example, from wood and cereal straw, entirely of the two compounds without breaking down the cellulosic constituent to a considerable extent.

Complete hydrolysis of cellulose yields glucose only. With the reservation that some of the glucose is destroyed under the influence of the hydrolyzing acid and some undergoes reversion, the yield approaches the theoretical. On gradual and carefully controlled hydrolysis or acetolysis (i.e., hydrolysis with simultaneous acetylation) a number of oligosaccharides, which consist of six, four, and three glucose anhydride units, may be obtained before the chains break down to the disaccharide, cellobiose, and to the end product, glucose. Whereas it has not yet been possible to unite glucose anhydride molecules to form chains containing more than three members by common laboratory methods, long glucose anhydride chains result from simple sugars by enzymic synthesis. The product of reaction, bacterial cellulose (B-cellulose) has been found to possess the molecular and submicroscopical structure of natural plant cellulose.[1]

[1] Hibbert and Barsha, Can. J. Research, 5, 580 (1931); Clark, "Applied X-Rays," McGraw-Hill Book Co., New York (1940), 3rd ed., p. 605.

The condensed formula of cellulose is usually written as $(C_6H_{10}O_5)_n$. On the assumption that the chain is open and thus possesses two terminating units which are different from the rest, the condensed formula should be written thus:

$$C_6H_{11}O_6—[C_6H_{10}O_5]_n—C_6H_{11}O_5$$

Cellulose possesses a high molecular weight. On the assumption that cellulose is represented by a system of polymeric molecules without side chains its molecular weight corresponds to the number of glucose anhydride units of the chain molecules multiplied by the molecular weight of the unit (162). The number of units, that is the number of times the building unit repeats itself in the chain molecule, is termed the degree of polymerization. Since the chains constituting the cellulose substance are not all of the same length, there can be only an average degree of polymerization and an average molecular weight.

A simple and approximate assessment of the degree of polymerization of cellulosic materials may be made by determining their solution viscosities. The viscosity decreases with decreasing degree of polymerization and may thus be used for recognizing and controlling the amount of degradation which cellulosic materials undergo during the various steps of isolation, purification, conversion into derivatives and during other reactions. This and other methods of determining the molecular weight of cellulose will be discussed later.

THE FORMATION OF ALKALI CELLULOSE AND CELLULOSATES

Caustic soda solution containing about 18 per cent sodium hydroxide by weight exerts a considerable swelling effect upon cellulose, an effect which is commercially utilized in the process of mercerization. This phenomenon is distinctly exothermic, and it seems likely that, under the conditions of mercerization, the two components combine to form a definite chemical compound. Its composition may be either $(C_6H_{10}O_5)_2 \cdot$ NaOH or $C_6H_{10}O_5 \cdot NaOH$,* depending upon the method of analysis applied. The compound is termed alkali cellulose or, more exactly, sodium hydroxide cellulose.

The combination of two, or one, glucose anhydride units with only one molecule of sodium hydroxide has its parallel in the behavior of a number of simple alcohols, polyalcohols, and polyhydroxy compounds with metal hydroxides. Thus, for example, glycerol, erythritol, mannitol, and dulcitol, as well as di- and monosaccharides combine with

* This formula expresses the fact that the compound contains one molecule of sodium hydroxide per glucose anhydride unit of the cellulose chain. In this and like formulas throughout the chapter, the polymeric nature of the cellulose portion is implied.

metal hydroxides in varying proportions to form addition compounds which may be regarded as complex compounds in the Werner sense. Whether the addition of sodium hydroxide to cellulose is governed by the same laws which seem to control the combination in the case of the simpler alcohols and sugars, is a question which is difficult to decide. In view of the complex nature of the cellulose system, its combination with alkali is more probably a matter of accessibility of hydroxyl groups. On the assumption that about half of the hydroxyl groups of the system are exposed on the surfaces of the micellae or chain bundles whereas the other half are concealed in the interior of the micellar system,[4] it is conceivable that the hydroxyl groups on the surface react first. If the reaction, as is actually the case, comes to a standstill, it is possibly because all the surface hydroxyls are covered. On this assumption, the formula, in which one molecule of sodium hydroxide corresponds to two glucose residues, might well be interpreted to indicate that such a type of surface reaction takes place and that the components are present not in a stoichiometric but rather in a pseudostoichiometric proportion.

The reaction between cellulose and strong sodium hydroxide solution may also be looked upon as comparable to alcoholate formation. This concept appears to derive support, although indirectly, from the so-called viscose reaction, i.e., the conversion of alkali cellulose into sodium cellulose xanthate (see later). This reaction, in its turn, appears to be analogous to the formation of sodium ethyl xanthate from sodium hydroxide dissolved in ethanol or from sodium ethoxide as such and carbon disulfide. As a matter of fact, metallic sodium dissolved in liquid ammonia reacts with cellulose with the liberation of hydrogen to form sodium cellulosates in which one, two, or three hydrogen atoms per glucose residue are replaced by sodium.[5]

It is interesting to note that the mercerizing effect usually secured at about 20° with a 17–18 per cent caustic soda solution is obtained at lower temperatures with less concentrated solutions; for example, at −10° a concentration of 6.5 per cent suffices. Whether the low-temperature combination leads to the same alkali compound as under normal conditions cannot yet be answered with certainty.

The theory of a number of investigators that the reaction between cellulose and sodium hydroxide is one of simple adsorption according to purely physical laws [6] is not verified by x-ray analysis (p. 1709), for in

[4] Meyer, Z. angew. Chem., **41**, 935 (1928); Mark and Meyer, Cellulosechem., **11**, 99 (1930).

[5] Scherer and Hussey, J. Am. Chem. Soc., **53**, 2344 (1931); Schorigin and Makarowa-Semljanskaja, Ber., **69**, 1713 (1936).

[6] Bancroft and Calkin, J. Phys. Chem., **39**, 1 (1935); Calkin, "Colloid Symposium Monograph" (1936); J. Phys. Chem., **40**, 38 (1936); Bancroft, ibid., **40**, 44 (1936).

following the reaction, using sodium hydroxide of increasing concentration, x-ray analysis shows that true adsorption is encountered only below a concentration of about 9 per cent. Below this concentration the x-ray pattern of the fiber remains unchanged, but with increasing concentration the diagram begins to change and shows a very distinct alteration at a concentration in the neighborhood of 17 per cent.[7]

Alkali celluloses may also be obtained with the hydroxides of potassium, lithium, cesium, and rubidium. Cellulose combines with the first two in the same proportion as with sodium hydroxide, but it takes up less alkali hydroxide in the case of cesium and rubidium. It is interesting to note that each hydroxide shows its maximum swelling effect upon cellulose at a definite concentration. This effect follows the order of the Hoffmeister series, i.e., it is greatest with lithium hydroxide, less pronounced with sodium and potassium hydroxide, and smallest with cesium hydroxide, and in each instance the maximum coincides with that concentration of the hydroxide solution at which formation of the alkali cellulose compound occurs.[8] This coincidence shows the beneficial effect which is realized if cellulose is made to swell, its reactivity being increased considerably. In some cases, a certain degree of swelling is indispensable for enabling the reagents to penetrate even the surface of the fiber.

Alkali cellulose is very unstable, being easily decomposed by water into its components. The cellulose is regenerated with a number of its physical properties changed. The term "cellulose hydrate" for the regenerated (mercerized) cellulose is derived from the earlier conception that the cellulose is regenerated from alkali cellulose with one molecule of water chemically combined. This, however, is not the case. The physical changes, due principally to swelling under the influence of alkalies, are indicated particularly by increased hygroscopicity, greater absorptive capacity for dyestuffs, and greater reactivity in general, provided that the cellulose hydrate is not subjected to much drying. This increased reactivity of cellulose hydrate has also been demonstrated by the intensified action of enzymes, such as cellulase, which leads to the formation of glucose.[9]

As stated before, the physical changes are also reflected in the x-ray diagram of the cellulose hydrates; the lattice appears slightly deformed

[7] Katz and Mark, Z. Elektrochem., 31, 105 (1925); Katz and Vieweg, ibid., 31, 157 (1925); v. Susich and Wolff, Z. physik. Chem., B8, 221 (1930); Schramek and Schubert, Z. physik. Chem., B13, 462 (1931).

[8] Heuser and Bartunek, Cellulosechem., 6, 19 (1925).

[9] Karrer and Illing, Helv. Chim. Acta, 8, 245 (1925); Karrer, Schubert, and Wehrli, ibid., 8, 797 (1925).

and widened, which might explain the greater reactivity of cellulose hydrate in comparison with native cellulose.[10]

The same physical changes are observed in all cellulose preparations which are obtained by regeneration, for example, from solutions of cellulose in cuprammonium hydroxide and from esters. In the latter case, however, the extent of the changes depends upon the method of esterification.

The mercerizing effect (i.e., the phenomenon in its physical aspect) is also obtained by allowing strong inorganic acids, such as concentrated sulfuric or nitric acid, to act upon cellulose for a short time. Here again, the result is due to the swelling effect which these acids exert.

From x-ray analysis various types of alkali cellulose seem to exist, and by certain treatments it has been possible to revert the regenerated cellulose, i.e., the hydrate modification, into its original native form.[11]

A number of strong organic bases, such as trimethylsulfonium hydroxide and guanidinium hydroxide and quaternary ammonium bases,[12] as well as ammonia,[13] also combine with cellulose in varying proportions. Some of the quaternary ammonium bases such as, for example, trimethylbenzylammonium hydroxide are able to dissolve cellulose.[14]

Cellulose, steeped in sodium hydroxide solution of mercerizing strength and freed of most of the excess alkali by pressing or centrifuging, undergoes certain chemical changes when allowed to stand for a longer period of time. These changes which are grouped under the term "aging" chiefly consist of a decrease in the solution viscosity of the regenerated cellulose, e.g., in cuprammonium solution, and an increase in the solubility of the regenerate in an 8 per cent caustic soda solution.[15] Besides, the reducing power first increases, then decreases, while the methylene blue absorption, being indicative of the presence of carboxylic groups, increases. These changes and the fact that they are much less pronounced when aging takes place with the exclusion of air and are more marked when oxygen is given access, indicate that aging is a process of oxidation.[16]

[10] Mark, "Physik und Chemie der Cellulose," Springer, Berlin (1932), p. 215; Sisson, *J. Phys. Chem.*, **40**, 343 (1936); *J. Am. Chem. Soc.*, **58**, 1635 (1936); Mark and Kratky, *Z. physik. Chem.*, **B36**, 130 (1937).

[11] Hess and Gundermann, *Ber.*, **70**, 527 (1937); Sobue, Kiessig, and Hess, *Z. physik. Chem.*, **B42**, 309 (1939); see also Schramek and Küttner, *Kolloid-Beihefte*, **42**, 331 (1935).

[12] Dehnert and Konig, *Cellulosechem.*, **6**, 1 (1925); Sisson and Saner, *J. Phys. Chem.*, **43**, 687 (1939).

[13] Barry, Peterson, and King, *J. Am. Chem. Soc.*, **58**, 333 (1936); Clark and Parker, *J. Phys. Chem.*, **41**, 777 (1937); Hess and Trogus, *Ber.*, **68**, 1986 (1935).

[14] Lieser, *Ann.*, **528**, 276 (1937); Bock, *Ind. Eng. Chem.*, **29**, 965 (1937).

[15] Heuser and Schuster, *Cellulosechem.*, **7**, 17 (1926); Waentig, *Kolloid-Z.*, **41**, 154 (1927).

[16] Weltzien and zum Tobel, *Ber.*, **60**, 2024 (1927); Lottermoser and Schwarz, *Kolloid-Beihefte*, **42**, 406 (1935); Davidson, *J. Textile Inst.*, **23**, T95 (1932); *ibid.*, **29**, T27 and T215 (1938).

Both the decreased viscosity and the increased solubility of the aged alkali cellulose show that oxidation has led to cleavage of glycosidic linkages, i.e., the chain molecules have been shortened. Since very little oxygen is required for changing the viscosity and solubility considerably, it is likely that oxidation attacks the chains at the center rather than at the ends.[17] On the assumption that primary valence cross linkages exist between individual chain molecules, it is possible that part or all of these cross linkages are also broken down either prior to or simultaneously with the attack upon the glycosidic linkages.

The effect of aging is appreciable only if the aqueous alkali used approaches mercerizing strength. Under these conditions cellulose swells considerably and acquires, thus, a highly reactive state. Whether the high alkali concentration is also essential for other reasons cannot be said with certainty. On the other hand, the aging effect is much reduced if cellulose is allowed to stand merely covered with strong aqueous alkali. Obviously, the layer of liquid prevents the air from entering the fiber from the outside. Besides, under these conditions, the microscopic and submicroscopic capillaries of the fibrous system are filled with liquid which prevents atmospheric oxygen from attacking the inner surface of the fiber or delays this process considerably.

Hot alkalies do not exert the mercerizing effect; they dissolve cellulose partly or completely, depending upon concentration, temperature, and pressure. On complete solution at high temperature and under high pressure, a variety of decomposition products of various sugars results, among which is found principally lactic acid.[18] W. L. Evans and his school have thrown much light on the mechanism of alkaline degradation of simple sugars and various di- and oligosaccharides.[19]

Fusion of cellulose with solid alkali hydroxide results in far-reaching degradation. The chief product, as has been known for a long time, is oxalic acid.* The mechanism of this reaction has been studied more recently by Fry and Otto.[20]

[17] Staudinger and Husemann, *Ber.*, **71**, 1059 (1938); Staudinger and Jurisch, *Ber.*, **71**, 2283 (1938).

[18] Odén and Lindberg, *Ind. Eng. Chem.*, **19**, 132 (1927); Heuser, *Paper Trade J.*, **89**, No. 26, 67 (1929).

[19] Evans and collaborators, *J. Am. Chem. Soc.*, **53**, 4384 (1931); **54**, 698 (1932); Plunkett and Evans, *ibid.*, **60**, 2847 (1938). See, also, Spengler and Pfannenstiel, *Angew. Chem.*, **48**, 475 (1935).

[20] Fry and Otto, *J. Am. Chem. Soc.*, **50**, 1138 (1928).

CUPRAMMONIUM CELLULOSE

Cellulose first swells considerably, then disperses (dissolves) in a solution of cupric oxide in ammonia. This solvent, called "Schweizer's reagent" or "cuprammonium" solution, is one of the very few solvents for cellulose from which it may be regenerated practically unchanged chemically, provided that air and light are excluded.

It will be remembered that the complex base, copper-tetraammino hydroxide $[Cu(NH_3)_4(OH)_2]$, contained in the cuprammonium solution, reacts with some polyalcohols like glycerol to form complex compounds. Cellulose, too, is capable of forming a complex addition compound with the base, in which possibly two glucose units react with one copper atom to form a cellulosate, which then unites with one molecule of copper-tetraammino hydroxide to form copper-tetraammino copper cellulose. The result of this reaction may be expressed by the hypothetical formula $[(C_6H_8O_5)_2Cu]\cdot[Cu(NH_3)_4]$. In this compound the portion $[(C_6H_8O_5)_2Cu]$ represents a complex anion, showing a ratio of cellulose (glucose units) to copper of 2 : 1.

Cellulose in cuprammonium solution shows pronounced levorotation which, according to Hess and Messmer,[21] is due to the presence of the complex cellulose copper anion. A change of the rotation value indicates a change in the concentration of this optically active anion. Evaluation of the experimental data in accordance with the law of mass action makes a 1 : 1 ratio of cellulose to copper in the complex anion probable. Therefore, the complex cellulose copper compound as it exists in solution has been given the formula $[C_6H_7O_5Cu]_2\cdot[Cu(NH_3)_4]$. It has not yet been possible to isolate this compound. However, if instead of copper-tetraammino hydroxide, copper-ethylenediamine hydroxide is used, which, as Traube[22] has shown, also dissolves cellulose, a complex compound may be isolated. Analysis of this compound justifies the formula $[(C_6H_8O_5)_2Cu]\cdot[Cu(En)_2]$ in which "En" represents ethylenediamine and which shows that in the hypothetical copper-tetraammino formula the four ammonia molecules are replaced by two ethylenediamine molecules.

Both the copper-tetraammino and the copper-ethylenediamine compound form complex metal salts. With sodium hydroxide, for example, a compound of the formula $[(C_6H_8O_5)_2Cu]\cdot Na_2$ is obtained which, after its discoverer, is also known under the term "Normann com-

[21] Hess and Messmer, *Ber.*, **54**, 834 (1921); **55**, 2441 (1922); **56**, 587 (1923); *Z. physik. Chem.*, **146**, 430 (1929); see, also, Hess, "Die Chemie der Cellulose," Akad. Verlags-Ges. Leipzig (1928), p. 294.

[22] Traube, *Ber.*, **44**, 3319 (1911); Traube and Funk, *Ber.*, **69**, 1476 (1936).

pound." [23] Corresponding compounds are formed with the alkaline earths and with thallium nitrate. [24]

Cellulose may be regenerated from its solution in cuprammonium hydroxide by means of alcohols, dilute acids, ammonium chloride, and many other salts, as well as by alkalies. No chemical change can be recognized, provided that regeneration is brought about not too long after dissolution and that the dissolution took place with careful exclusion of oxygen (air) and light. [25] In the presence of air, cellulose dissolved in cuprammonium solution is very sensitive and undergoes partial oxidation. This change, which is enhanced by light and which under these conditions becomes measurable after only a few minutes, is indicated by a decrease in the viscosity and an increase in the solubility of the regenerate in 8 per cent caustic soda solution.

Cuprammonium solution, because of its pronounced solvent power upon cellulose and the fact that it does not degrade it if proper precautions are observed, is the most suitable solvent for any cellulose preparation the viscosity of which is to be determined. [26]

Cellulose may also be dissolved in solutions of a number of electrolytes, particularly in those which exert a pronounced swelling effect upon it, as, for example, the thiocyanates. Usually, an elevated temperature is required to achieve dissolution, and the cellulose is more or less degraded. [27] Recently some mineral acids (particularly phosphoric acid) have been described as good actual solvents for cellulose, when used with proper precautions. [28] The use of quaternary ammonium bases as solvents has been mentioned before. [14]

[23] Normann, Chem. Ztg., 30, 584 (1906); Hess and Messmer, Ber., 55, 2432 (1922); Traube, Ber., 55, 1899 (1922); Heuser and Brötz, Papier-Fabr., 25, 238 (1927); Hess and Trogus, Z. physik. Chem., A145, 401 (1929).

[24] Traube and Funk, Ber., 69, 1476 (1936).

[25] Scheller, Melliand Textilber., 16, 787 (1935); see, also, Heuser, "Lehrbuch der Cellulosechemie," Borntraeger, Berlin (1927) 3rd ed., p. 176; Staudinger, "Die hochmolekularen organischen Verbindungen," Springer, Berlin (1932), pp. 494, 495; Bancroft and Ramsay, paper presented before the American Chemical Society Meeting at Pittsburgh, September, 1936.

[26] Standard method suggested by the American Chemical Society, Cellulose Division, Ind. Eng. Chem., Anal. Ed., 1, 49 (1929); Dorée, "The Methods of Cellulose Chemistry," Van Nostrand, New York (1933), p. 52.

[27] von Weimarn, Kolloid-Z., 11, 41 (1912); 29, 197 (1921); 36, 103 (1925); Steingroever, Cellulosechem., 8, 37 (1927); Herzog and Beck, Z. physiol. Chem., 111, 287 (1920); Herzog, Kolloid-Z., 39, 98 (1926); Staudinger, "Die hochmolekularen organischen Verbindungen," p. 492; Erbring and Geinitz, Kolloid-Z., 84, 25 (1938).

[28] af Ekenstam, Ber., 69, 549, 553 (1936).

CELLULOSE ESTERS

Just as an alcohol undergoes esterification with an acid in the presence of a dehydrating agent or by the action of an acid chloride, so cellulose may be converted into esters. It has not always been possible to convert all three hydroxyls into ester groups, and esterification may arrest itself at two hydroxyls and sometimes even at one. Again, such a course of the reaction is due to the peculiarities of the microscopic and submicroscopic structure of the fiber which causes the hydroxyl groups of the chain bundles to be not equally accessible. Moreover, the fact that the three hydroxyl groups available in each glucose residue are not equivalent may also influence the course of the reaction. To facilitate the access of the agents and thus to complete the reaction within reasonable time, esterification is allowed to take place in the presence of swelling agents such as a strong mineral acid or certain salts. In commercial practice these agents are known as "catalysts." Under such conditions, however, cellulose becomes considerably degraded, particularly if esterification is allowed to take place at elevated temperature. The result of esterification, then, is a mixture of molecules of greater and smaller chain length, among which the esters of even oligosaccharides, cellobiose, and glucose may be found. If degradation is to be avoided, the use of degrading swelling agents must be omitted, in which case a very long time is required for completing the reaction.

The cellulose esters are soluble in organic solvents, each ester having one (or more) most suitable solvent or mixture of solvents depending upon the balance of polar and nonpolar groups present in the ester and similar groups present in the solvent or solvent mixture.[29] The extent to which the esters dissolve may often be used as an indication of both the degree of substitution and the degree of polymerization. The viscosity of the solution is dependent primarily upon the extent of degradation which the cellulose has undergone on esterification, low viscosity indicating a far-reaching degradation, and vice versa. In addition, the degree of substitution may be a governing factor.

The cellulose esters, like aliphatic esters, yield to saponification, whereupon cellulose and acid are obtained. Sometimes it is possible to regenerate the cellulose without further degradation, and in this case an investigation of the regenerate (e.g., by determining its viscosity in cuprammonium solution) shows how much degradation the cellulose has suffered during esterification.

[29] Highfield, *Trans. Faraday Soc.*, **22**, 57 (1926) ; *Z. physik. Chem.*, **124**, 245 (1926).

Cellulose nitrates (nitrocellulose, nitric acid esters) may be obtained by treating cellulose with a mixture of nitric and sulfuric acid or other mineral acids containing a certain percentage of water, at a temperature preferably not higher than 40°. The reaction may be expressed by the following equation:

$$C_6H_9O_4OH + HONO_2 \rightleftarrows C_6H_9O_4ONO_2 + H_2O$$
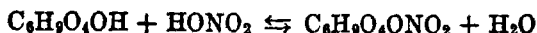
Of the factors governing the process, the water content of the acid mixture is of predominating influence upon the characteristics of the nitrocellulose, particularly upon its nitrogen content and its solubility in certain solvents, such as ether-alcohol mixtures. The use of higher temperature, although being of favorable influence upon the velocity of reaction, would be detrimental, considering the fact that the acid mixture not only brings about esterification but at the same time exerts a hydrolyzing and oxidizing effect upon cellulose; besides, the acid tends to hydrolyze the ester. The results of the side reactions are impurities which have to be removed in order to obtain a stable ester. Purification ("stabilization") may be accomplished by boiling the nitrate with water for many hours. This treatment also removes the residue of sulfuric acid present in the unpurified nitrate in the form of cellulose sulfates. On standing, the sulfates tend to become hydrolyzed, and the presence of free sulfuric acid gives rise to decomposition of the nitrate. Drying of nitrocellulose should be avoided, since it is easily inflammable. The higher nitrates, if detonated by a blow or by the aid of mercury fulminate, are highly explosive.

The nitration acid displays its maximum power of substitution if the nitric acid is present as a 100 per cent HNO_3. The dehydrating properties of sulfuric acid are utilized in the acid mixture for approaching this concentration of the nitric acid since the sulfuric acid takes up both the free water in the system and the water formed on reaction. Although nitration may be accomplished by water-free nitric acid,[30] water is used for maintaining a high acid ratio (efficient diffusion) in a more economical way.

Sulfuric acid may be replaced by phosphoric[31] or by glacial acetic acid.[32] Phosphoric acid has been found to yield cellulose trinitrate of the theoretical nitrogen content, 14.17 per cent, $C_6H_7O_2(ONO_2)_3$, which is not obtainable with sulfuric acid as the dehydrating agent because of the

[30] Rogovin and Tichonow, *Cellulosechem.*, **15**, 102 (1934); Dalmon, Chédin, and Brissaud, *Compt. rend.*, **201**, 664 (1935).

[31] Lunge and Weintraub, *Z. angew. Chem.*, **12**, 445 (1899); Berl and Rueff, *Cellulosechem.*, **14**, 115 (1933).

[32] Trogus, *Ber.*, **64**, 405 (1931).

tendency of the latter system to establish equilibrium between esterification and hydrolysis.

The products of the lower stages of substitution, which are of commercial interest, may be prepared by increasing the water content of the acid mixture over that required for preparing the trinitrate.[82]

On nitration cellulose retains its fibrous structure, for the nitration reaction proceeds at a rather fast rate and thus prevents the acids from peptizing and dispersing the fiber. In addition, the entry of a nitrate group stabilizes the cellulosic constituent against degradation. The velocity of reaction, which sometimes may require only seconds for reaching a relatively high nitrogen content, would permit the conclusion that the reaction advances quickly through the entire inter- and intra-micellar structure of the fiber in a "permutoidal" fashion. This type of reaction seems to lead to a rather uniform distribution of nitrate groups over all chain molecules. However, the incompletely nitrated product is evidently not a mixture of individual mono- and disubstituted with trisubstituted cellulose, for it has not been possible to separate an incompletely substituted nitrate into fractions of substantially different nitrogen content.

By analogy with results obtained in the methyl glucoside series only nitrate groups in the sixth position are considered to be replaced by iodine when nitrocellulose is subjected to the action of anhydrous sodium iodide.[84] When this reaction was allowed to take place with low-substituted nitrates (2.5–6.1 per cent nitrogen content) it was found that at least 44 per cent of the nitration had occurred in the 6-position. This result might be interpreted to mean that the primary alcohol groups of cellulose react at a faster rate than the secondary groups.

In contrast to the constancy of the nitrogen content, nitrocellulose fractions which may be prepared according to various techniques vary considerably as to viscosity and other physical properties, which shows nitrocellulose to be rather heterogeneous in respect to chain length. The degree of homogeneity is a commercially important characteristic since it influences the properties of the products (films, etc.) prepared from nitrocellulose.[85]

With nitric acid of lower concentration (62–68.6 per cent) than required for nitration (75–77 per cent), cellulose forms an addition compound which, because of its discoverer, is called "Knecht's compound" and which has been given the formula $C_6H_{10}O_5 \cdot HNO_3 \cdot H_2O$. Its first appearance may be observed by x-ray photography.[86]

[82] Berl, Andress, and Escales, *Kunststoffe*, **27**, 23 (1937).
[84] Murray and Purves, *J. Am. Chem. Soc.*, **62**, 3194 (1940).
[85] Spurlin, *Ind. Eng. Chem.*, **30**, 538 (1938).
[86] Trogus, *Cellulosechem.*, **15**, 104 (1934).

X-ray analysis also allows the process of nitration to be followed to a certain extent. Obviously, only the trinitrate possesses an ordered, i.e., crystalline, lattice structure whereas the intermediate stages are capable of yielding only an amorphous pattern.[37]

The nitric acid esters may be hydrolyzed by means of strong sulfuric acid. The inorganic group is quantitatively regenerated as nitric acid, while the cellulose is largely degraded. Such degradation also occurs with most of the other methods in which the nitrogen may be liberated as such, or in the form of nitric acid, nitric oxide, or other nitrogen compounds.

On saponification with aqueous alkali, whereby most of the nitrogen is recovered as nitrite, the cellulose becomes oxidized by the oxygen of the nitrate groups.[38] However, with potassium or ammonium hydrosulfide which also convert all the nitrogen into nitrite, cellulose emerges with somewhat less degradation.[39]

Nitrocellulose is used commercially, among other purposes, for the manufacture of smokeless gun powder, in films for motion pictures, and for plastics, such as celluloid, which is a mixture of nitrocellulose and camphor. The manufacture of rayon from nitrocellulose was abandoned in this country a few years ago. Rayon from nitrocellulose was the first artificial cellulose fiber commercially produced (Count de Chardonnet, 1884).

In regard to cellulose sulfates the reader may be referred to the literature references below.[40]

Cellulose acetates are usually obtained on treatment of cellulose with a mixture of acetic anhydride and glacial acetic acid in the presence of agents which exert a marked swelling action upon cellulose. The acetic acid acts as a diluent and solvent for the acetate. Sulfuric acid, sulfuryl chloride, zinc chloride, perchloric acid, and many other "catalysts" may be used. Simultaneously these agents exert a degrading effect upon cellulose, which is enhanced at elevated temperature. Under the influence of the reagents cellulose gradually loses its fibrous structure, passing into the state of a thick paste and finally into a viscous dispersion (solution). After acetylation is completed, the solution is poured into water whereby the acids and most of the water-soluble products of degradation are removed and the acetate is obtained in the form of white

[37] Sisson, *Ind. Eng. Chem.*, **30**, 530 (1938).
[38] Kenyon and Gray, *J. Am. Chem. Soc.*, **58**, 1422 (1936).
[39] Rassow and Dörr, *J. prakt. Chem.*, **108**, 113 (1924); Staudinger and Mohr, *Ber.*, **70**, 2306 (1937).
[40] Heuser, "Lehrbuch der Cellulosechemie," p. 54; Traube, Blaser, and Grunert, *Ber.*, **61**, 754 (1928); **65**, 603 (1932); Gebauer-Füllnegg, Stevens, and Dingler, *Ber.*, **61**, 2000 (1928).

flocks. When sulfuric acid is used as "catalyst," the ester still contains a certain, though small, amount of combined sulfuric acid which, however, is reduced to a fraction of a tenth of one per cent on boiling in water ("stabilization").

Glacial acetic acid alone has very little effect on cotton cellulose at room temperature, but on boiling, a few per cent of acetyl groups may be introduced. This amount may be considerably increased with cellulosic materials of lower degree of polymerization and of greater reactivity.[41, 42]

Under similar conditions acetic anhydride (without the presence of a catalyst) is more potent. Thus, it has been possible to prepare an acetate with 54 per cent combined acetic acid from mercerized cotton cellulose by boiling with acetic anhydride for 580 hours.[42]

Although they retain their fibrous structure the products of reaction suffer a great deal of degradation under such severe conditions.

Acetylation in the presence of "catalysts" proceeds at a considerably slower rate than nitration, the reaction being more of a topochemical nature.[43] The intermediates which may be isolated after certain intervals during the earlier stages of esterification are mixtures of fully and partly acetylated chains with others not yet acetylated. However, as the mixture approaches the state of solution the reaction becomes more homogeneous, and substitution proceeds more uniformly until the triacetate is reached. Thus, only the triacetate, after the water-soluble esters have been removed, may be regarded as uniform as far as substitution is concerned[44] (theoretically required for $C_6H_7O_2(OCOCH_3)_3$, 44.8 per cent acetyl or 62.5 per cent combined acetic acid). However, in all acetates which are prepared in the presence of auxiliary agents of the type mentioned above, the cellulosic constituent is degraded to an average chain length far below that of the original cellulosic material.

By diluting the acetic anhydride–sulfuric acid mixture with benzene or carbon tetrachloride, i.e., liquids in which the acetate is insoluble and which obviate the swelling effect of the catalyst to a considerable extent, the fibrous structure of the original cellulose is maintained. Thus a product suitable for making x-ray fiber diagrams is obtained and, as in nitration, acetylation may be followed by x-ray analysis of samples taken at intervals. As on nitration, the x-ray pattern of the original cellulose does not change until a rather high combined acetic acid content has been reached. This state is followed by the appearance of

[41] Cross, Bevan, and Traquair, Chem. Ztg., 29, 528 (1905); Malm and Clarke, J. Am. Chem. Soc., 51, 274 (1929).

[42] Bernoulli, Schenk, and Hagenbuch, Helv. Chim. Acta, 13, 539, 550, 557 (1930).

[43] Kanamaru, ibid., 17, 1429 (1934).

[44] Ost, Z. angew. Chem., 32, 66, 76, 82 (1919).

a mixed cellulose-cellulose triacetate pattern which gradually gives way to the exclusive triacetate diagram. Another way of following the process of acetylation, also adaptable to nitration,[43] is to observe the change in double refraction of the fibers undergoing esterification.[45]

Whereas the cellulose constituent in fibrous acetates prepared in the way indicated above is still degraded, although less than on acetylation in solution, practically undegraded fibrous acetates may be obtained by omitting the sulfuric acid (or other degrading catalysts) and, instead, applying acetic anhydride alone, dissolved in pyridine.[46]

Incompletely substituted acetates with their acetyl groups more uniformly distributed than in products attainable by incomplete acetylation may be obtained by "partial hydrolysis" * of the triacetate, for instance, with dilute mineral acids according to a process suggested by Miles for commercial purposes or by modifications thereof.[47] Such partially "saponified" ("secondary") acetates (varying from 55 to 59 per cent combined acetic acid content) are soluble in acetone in contrast to cellulose triacetate which is insoluble in this cheap and practical solvent. From a commercial point of view, acetone-solubility is of considerable importance; it is essentially a function of the acetyl content but seems to be governed also by the distribution of covered and uncovered hydroxyl groups. By allowing p-toluenesulfonyl chloride to act upon a commercial secondary acetate and by subsequent replacement of part of the tosyl † groups by iodine or chlorine, Cramer and Purves [48] found that partial saponification involved the removal of acetyl groups from primary and secondary positions in approximately the same proportion. On reacetylation a triacetate is obtained which again is insoluble in acetone. The introduction of tosyl groups into secondary cellulose acetate proceeds rather rapidly at first but slows down very definitely after some time. The application of the iodine reaction to a tosyl cellulose acetate which had been isolated at this point, i.e., after about one-third of the free hydroxyl groups of the secondary acetate had been tosylated, showed that at least 84 to 90 per cent of the uncovered hydroxyl groups of the secondary acetate were in the primary position. Possibly the

[43] Möhring, Wiss. Ind., 1, 70 (1923); Spence, J. Phys. Chem., 43, 865 (1939).

[45] Hess and Ljubitsch, Ber., 61, 1460 (1928); Staudinger and Schweitzer, Ber., 63, 3132 (1930); Staudinger and Daumiller, Ann., 529, 219 (1937).

* In the German literature the term "saponification" is used regardless of whether the hydrolyzing agent is an aqueous acid or an aqueous alkali.

[47] Miles, U. S. pat. 838,350 (1904); Ost, Z. angew. Chem., 32, 66, 72, 82 (1919); Elöd and Schrodt, Z. angew. Chem., 44, 933 (1931).

† According to a suggestion by Hess and Pfleger, Ann., 507, 48 (1933), "tosyl" is used as an abbreviation for p-toluenesulfonyl.

[48] Cramer and Purves, J. Am. Chem. Soc., 61, 3458 (1939); see, also, Cramer, Hockett, and Purves, ibid., 61, 3463 (1939).

presence in a secondary acetate of a certain number of free primary hydroxyl groups is essential for causing solubility in acetone.

The most common solvent for cellulose triacetate is chloroform or a mixture of chloroform and alcohol. Such a solution (as well as solutions of cellulose triacetate in tetrachloroethane and in pyridine) show optical levorotation, which, however, inverts into dextrorotation when acetylation is carried to the state of acetolysis.[49]

Complete hydrolysis, which may be brought about by means of acids or alkalies, regenerates the acetic acid (which may be determined analytically [50]) and the cellulose in a more or less degraded form, depending upon the agent used and the conditions chosen. However, degradation during regeneration may be avoided by applying an alcoholic solution of sodium ethoxide [51] or a methyl alcoholic sodium hydroxide solution in a nitrogen atmosphere, or by dissolving the acetate in cuprammonium solution, again with the exclusion of air.[52]

The cellulose acetates are of considerable commercial interest. Their properties vary with the degree of polymerization and the degree of substitution. They are used chiefly for the manufacture of lacquers, plastics, and acetate rayon.[53]

Of other organic acids,[54] the following have been more or less successfully used for esterification of cellulose: formic, chloroacetic, carbonic, propionic, butyric, stearic, lauric, palmitic, caprylic, and oxalic and other dibasic acids; also aromatic acids, such as benzoic, phthalic, cinnamic, and a number of sulfonic acids, particularly p-toluenesulfonic acid.

Esters of p-toluenesulfonic acid, $C_6H_9O_4OSO_2C_6H_4CH_3$, are obtained by the action of the chloride of p-toluenesulfonic acid on cellulose in the presence of strong aqueous sodium hydroxide, pyridine, or other organic bases. The reaction may be represented by the following equation:

$$(C_6H_9O_4OH)_n + ClSO_2 \cdot C_6H_4CH_3 \rightarrow (C_6H_9O_4 \cdot SO_2 \cdot C_6H_4CH_3)_n + HCl$$

In order to reduce contamination of the ester with combined chlorine and nitrogen (if pyridine is used as a base) low esterification temperature has proved favorable. Under these conditions not more than two hydroxyl groups per glucose unit are replaced by tosyl groups, which

[49] Ost, *Z. angew. Chem.*, **32**, 67, 88 (1919).

[50] See the review by Genung and Mallatt, *Ind. Eng. Chem., Anal. Ed.*, **13**, 369 (1941).

[51] Zemplén, *Ber.*, **69**, 1827 (1936).

[52] Staudinger and Schweitzer, *Ber.*, **63**, 3132 (1930); Staudinger and Daumiller, *Ann.*, **529**, 256 (1937).

[53] Conaway, *Ind. Eng. Chem.*, **30**, 516 (1938); Ott, *ibid.*, **32**, 1641 (1940).

[54] Hess, "Die Chemie der Cellulose," p. 428; Marsh and Wood, "An Introduction to the Chemistry of Cellulose," Van Nostrand, New York (1939), p. 183; Malm and Fordyce, *Ind. Eng. Chem.*, **32**, 405 (1940).

may be interpreted to mean that two hydroxyl groups of the glucose residue are more easily replaced than the third.[55] Judging from the results obtained on the tosylation of secondary cellulose acetate discussed above it is likely that the primary alcoholic hydroxyl group is substituted first.

Under the influence of ammonia and aliphatic amines, part of the tosyl groups are removed, while the fiber takes up a small amount of nitrogen. The assumption that tosyl groups are thus replaced by amino groups[56] could not be confirmed. It is probable that the small amounts of chlorine contained in the tosyl ester are responsible for the combination with correspondingly small quantities of ammonia and amine.[57]

Mixed cellulose esters may be prepared by successive esterification with the respective agents. Nitrocellulose acetates and nitrocellulose benzoates have been known for some time. More recently, acetopropionates and acetobutyrates as well as acetophthalates have become of interest since their properties and compatibility with plasticizers seem to offer considerable commercial possibilities.[58]

Cellulose Xanthates. If carbon disulfide is allowed to act upon cellulose containing an excess of strong aqueous alkali a reaction takes place which may be expressed by the following equation:

$$R \cdot OH + NaOH + CS_2 \rightarrow \underset{\text{Cellulose}}{} \begin{array}{c} O \cdot R \\ | \\ C = S \\ | \\ SNa \end{array} + H_2O$$
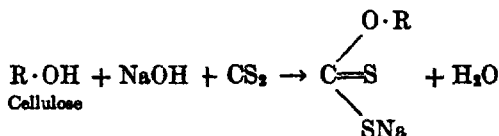
The product of reaction is the sodium salt of the dithiocarbonic acid ester of cellulose, sodium cellulose xanthate.

In this form the reaction is comparable with the formation of sodium ethyl xanthate which may be obtained by allowing carbon disulfide to react either directly with sodium ethoxide or with a solution of sodium hydroxide in ethyl alcohol. In the latter case we are dealing with an equilibrium reaction, thus:

$$C_2H_5OH + NaOH \rightleftarrows C_2H_5ONa + H_2O$$
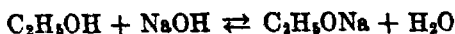
[55] Sakurada and Nakashima, *Sci. Papers Inst. Phys. Chem. Research (Tokyo)*, **6**, 214 (1927); Hess and Ljubitsch, *Ann.*, **507**, 62 (1933); Bernoulli and Stauffer, *Helv. Chim. Acta*, **23**, 627 (1940).

[56] Karrer and Wehrli, *Z. angew. Chem.*, **39**, 1509 (1926).

[57] Hess and Ljubitsch, *Ann.*, **507**, 68 (1933); see also Sakurada, *J. Soc. Chem. Ind. Japan*, supplemental binding, **32**, 11B (1929). Actually, aminocellulose has been prepared recently by allowing sodium amide to act on cellulose nitrate in liquid ammonia, Scherer and Feild, *Rayon Textile Monthly*, **22**, 607 (1941).

[58] Fordyce, Salo, and Clarke, *Ind. Eng. Chem.*, **28**, 1312 (1936); Fordyce and Meyer, *ibid.*, **32**, 1056 (1940).

but the decomposition of the alkoxide by the water formed is prevented by the presence of carbon disulfide because it quickly converts the sodium ethoxide into sodium ethyl xanthate, which is practically stable to water.

Likewise, the action of sodium hydroxide upon cellulose may be viewed as an equilibrium reaction, and, whereas this reaction tends to shift to the left, the presence of carbon disulfide will cause it to proceed in the opposite direction. In other words, small quantities of sodium cellulosate are continuously converted into sodium cellulose xanthate until the amount of carbon disulfide added is consumed.

Since cellulose xanthate formation proceeds at a relatively slow rate, the carbon disulfide has ample opportunity to react also with the aqueous sodium hydroxide to form sodium carbonate and sodium trithiocarbonate (Na_2CS_3). Thus, the yield of xanthate will depend upon which of the two competing reactions proceeds at the faster rate.

Cellulose in the absence of alkali does not react with carbon disulfide. Obviously, the formation of sodium cellulosate as a preliminary step is indispensable. As a matter of fact, the xanthate reaction takes place only if cellulose is treated with alkali of a concentration at which the alkali cellulose compound is formed.[59] Likewise, dimethyl cellulose, which does not combine with alkali, is unable to form a xanthate. However, methyl cellulose of a lower degree of substitution, which combines with alkali, undergoes the xanthate reaction.[59, 60]

The degree of substitution in cellulose xanthate depends essentially upon the amount of carbon disulfide applied and upon the degree of contact between the reactants. With a quantity of carbon disulfide corresponding to one to two molecules per $C_6H_{10}O_5$ unit, a xanthate is formed which carries one xanthate group per two glucose units, $NaS—CS—O(C_6H_9O_4)_2OH$.

This formula is confirmed by the fact that the 1:2 ratio of xanthate groups to glucose residues is maintained on converting the xanthate into a derivative, namely, by allowing diethylchloroacetamide [$ClCH_2CON(C_2H_5)_2$] to act upon the xanthate.[61] In this derivative, the composition of which justifies the formula

$$O(C_6H_9O_4)_2OH$$
$$\diagup$$
$$C{=}S$$
$$\diagdown$$
$$SCH_2CON(C_2H_5)_2$$
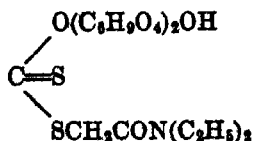
[59] Heuser and Schuster, *Cellulosechem.*, **7**, 29 (1926).
[60] Berl and Ritter, *ibid.*, **7**, 140 (1926).
[61] Fink, Stahn, and Matthes, *Angew. Chem.*, **47**, 602 (1934).

the ratio of the diethylacetamide radical (and the nitrogen content of the derivative) to glucose residues is again 1:2.

It is doubtful whether the composition of the xanthate having the ratio of one xanthate group per two glucose residues may be interpreted to mean that only surface-exposed hydroxyl groups are involved.[62,63] In view of the fact that the strong alkali used causes not only inter- but also intramicellar swelling it is very probable that inter- and intramicellar hydroxyl groups are involved in the formation of the alkali compound and that of the xanthate.

Although some evidence indicates that in the reacting glucose residues it is the hydroxyl group in the 2-position which undergoes the xanthate reaction, it is doubtful whether the reaction under conditions which result in a ratio of sodium to sulfur to cellulose of 1 : 2 : 2 is confined to this hydroxyl group.[64]

By employing a larger excess of carbon disulfide, particularly if such an excess is incorporated into the alkaline solution of a partially substituted xanthate, the degree of substitution may be raised to almost two xanthate groups per glucose unit.[65, 66] Moreover, cellulose dissolved in a quaternary ammonium base is capable of taking up nearly three xanthate groups per glucose unit.[64] It is significant that trisodium cellulosate, prepared by the action of sodium metal dissolved in liquid ammonia, under certain conditions, yields sodium cellulose trixanthate.[67]

The solution of cellulose xanthate in water or dilute alkali is termed viscose. It is of a colloidal nature and possesses a very high viscosity and an orange color, the color being due to contamination with sodium trithiocarbonate. The trixanthate is also soluble in methyl alcohol and in acetone.

The partially substituted xanthates may be precipitated from their solutions by means of alcohol or electrolytes, such as sodium or ammonium chloride or weak organic acids. Under the influence of dilute acetic acid the inorganic salts are decomposed whereas the xanthate is precipitated practically unchanged. With mineral acids cellulose xanthate is also precipitated but quickly decomposed into cellulose, carbon disulfide, and the sodium salt of the acid. The cellulose constituent emerges from this reaction in the form of cellulose hydrate, i.e., it shows the

[62] Lieser, *Ann.*, **470**, 104 (1929); **483**, 135 (1930).

[63] Schramek and Küttner, *Kolloid-Beihefte*, **42**, 221 (1935).

[64] Lieser and Leckzyck, *Ann.*, **522**, 56 (1936).

[65] Geiger, *Helv. Chim. Acta*, **13**, 287 (1930).

[66] Scherer and Miller, *Rayon Textile Monthly*, **19**, 478 (1938).

[67] Scherer, Gotsch, et al., *Bull. Virginia Polytech. Inst., Eng. Expt. Sta. Series Bull.*, No. 39, 3 (1939).

greater reactivity and the altered x-ray diagram of this cellulose modification. Besides, the regenerated cellulose possesses a lower degree of polymerization than the starting material which is due to the action of atmospheric oxygen during the preparation of the alkali cellulose, particularly if the latter was subjected to aging (see above) and during xanthation.[68, 69] If air is excluded from these processes, the cellulose constituent of the xanthate is not degraded.

On standing, viscose undergoes certain colloidal and chemical changes which are grouped under the term "ripening." The viscosity of the highly hydrated sol, which is high immediately after dissolving, gradually drops to a minimum, probably owing to a tendency of the originally coarse particles to become more fully dispersed and to undergo dehydration.[70] On further standing, the viscosity increases again, and, probably favored by the salting-out effect of the inorganic by-products. eventually becomes so high that it cannot be measured any longer. The system has developed a gel structure. On still longer standing, the gel undergoes syneresis. Viscose, thus, displays such colloidal changes as are characteristic of lyophilic colloids; they often occur without any change to the chemical composition of the colloid. In viscose, however, the colloidal changes are accompanied by a gradual hydrolysis of the xanthate which hastens the alteration of the colloidal state without necessarily being the only cause of the alteration.

Under the influence of hydrolysis the xanthate loses more and more of its xanthate groups. Sodium dithiocarbonate, which is possibly formed as an intermediate, decomposes into carbon disulfide and sodium hydroxide, which, in turn, react to form sodium carbonate and trithiocarbonate. Eventually, cellulose is regenerated, although it still contains a small quantity of sulfur. The ratio of sodium to sulfur to cellulose in the intermediate xanthates which may be isolated during ripening reflects the gradual loss of xanthate groups, but the ratio is not understood to indicate that the constituents are present in stoichiometric proportions.

The colloidal state of the solution during ripening is indicated by its behavior with electrolytes. The farther the ripening has proceeded, the less of a solution of an electrolyte is required to bring about coagulation of the dispersed compound.

The regenerated cellulose possesses practically the same degree of polymerization as the cellulose constituent of the xanthate before dis-

[68] Reference 59, p. 41.

[69] Lottermoser and Wultsch, *Kolloid-Z.*, **83**, 180 (1938).

[70] Mark, "Physik und Chemie der Cellulose," p. 259; Moore, *Silk and Rayon*, **8**, 507 (1934).

solving, which shows that, on ripening, the cellulose constituent of the xanthate is not degraded.[59, 69]

Commercially, viscose is used chiefly for the manufacture of cellophane and viscose rayon. About 80 per cent of all rayon is produced by this reaction, which was discovered by Cross and Bevan in 1893.

CELLULOSE ETHERS

Cellulose ethers may be obtained in the same way as aliphatic ethers, for example, by the action of alkyl halides or alkyl esters of inorganic acids upon alkali cellulose. What has been said with regard to the heterogeneous nature of the esterification reaction holds true also for the conversion of cellulose into its ethers. The mono- and diethers must be regarded as mixtures, and only the triether represents a homogeneous product. More recently, as a result of evidence from fractionation, it has been claimed that even trimethylcellulose is a mixture.[71]

It appears that cellulose is even more resistant to etherification than to esterification, and the reaction proceeds the more easily the more the cellulose has been degraded.[72] Thus cellulose regenerated from solutions or from processes of oxidation or from the action of acids may be converted into ethers within a shorter time than untreated native cellulose.[73]

Methyl and Ethyl Ethers. Methyl cellulose is of special scientific interest because, as will be seen later, it has greatly assisted the endeavors to elucidate the chemical constitution of cellulose. Both methyl and ethyl cellulose are prepared commercially and have found many uses.

The usual way of preparing methyl cellulose in the laboratory consists in allowing dimethyl sulfate to react with cellulose in the presence of strong sodium hydroxide solution at a temperature of about 50°.[74] The reaction, which may be represented by the following equation,

$$C_6H_9O_4 \cdot OH + (CH_3O)_2SO_2 \rightarrow C_6H_9O_4 \cdot O \cdot CH_3 + CH_3O \cdot SO_2OH$$
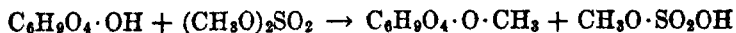
is exothermic, but the heat developed does not suffice to accelerate the reaction to any great extent. Usually more than one methylation is required to reach a high degree of substitution (the triether requires 45.57 per cent methoxyl).[75] Methylation may be facilitated if the hydroxyls of the cellulose are first converted into acetoxy groups. It appears feasible first to methylate to the dimethyl stage, which is

[71] Hess and collaborators, *Cellulosechem.*, **16**, 78 (1935).

[72] Conaway, *Ind. Eng. Chem.*, **30**, 516 (1938).

[73] Heuser and Hiemer, *Cellulosechem.*, **6**, 101, 125 (1925).

[74] Denham and Woodhouse, *J. Chem. Soc.*, **103**, 1735 (1913); **105**, 2357 (1914); **111**, 244 (1917); **119**, 81 (1921).

[75] Heuser and von Neuenstein, *Cellulosechem.*, **3**, 92 (1922); Irvine and Hirst, *J. Chem. Soc.*, **123**, 529 (1923).

comparatively easily reached, then to acetylate to dimethylmonoacetyl-
cellulose, and then methylate again, whereupon the acetyl is saponified
and replaced by the methyl group. The trimethylate thus obtained
contains 45.42 per cent methoxyl, but is that of a considerably degraded
cellulose.[75]

One may also subject the acetone solution of a secondary acetate to
the action of dimethyl sulfate and alkali.[76] Also this method facilitates
methylation, but in neither case has it been possible to reach the
theoretical value of the triether unless the acetate (or the cellulosic
material) used has a relatively low degree of polymerization.[77] Nor
does it seem to be possible to prepare trimethylcellulose, in which the
cellulose constituent is not degraded, by the action of methyl iodide on
trisodium cellulosate in liquid ammonia.[78]

Methyl cellulose is soluble in water to an extent depending upon its
degree of polymerization and its methoxyl content.[79] Products pre-
pared in the usual way, i.e., from fibrous cellulose or from an acetate,
which contain 1.5 to 2 methoxyls per glucose unit are soluble in cold
water. On heating they are precipitated, and on cooling they go into
solution again, which may be explained on the assumption that hydrates
are formed which suffer cleavage on heating.[78] Above the ratio of two
methoxyl groups per glucose residue the products become more and
more hydrophobic. On the other hand, if methylation is carried out on
cellulose dissolved in a quaternary ammonium base, as little as 0.7
methoxyl group per glucose residue (corresponding to about 12 per cent
methoxyl) suffices to make the product water-soluble. This result is
probably due to the fact that the reaction takes place in a more homo-
geneous system than with cellulose in fibrous form.[80]

The ethers of varying methoxyl content are soluble in pyridine,
chloroform, tetrachloroethane, etc., and show different levorotational
values in the various solutions.

The methyl groups may be liberated as from common aliphatic
ethers by allowing hydrogen iodide to act on the ether. The regenerated
cellulose is extensively degraded, however, as a result of the violence of
this reaction.

It is evident from x-ray analysis and from fractionation, as well as
from hydrolysis and acetolysis studies on incompletely methylated

[75] Haworth, Hirst, and Thomas, J. Chem. Soc., 821 (1931); Haworth and Machemer, ibid., 2270 (1932).
[77] Karrer and Escher, Helv. Chim. Acta, 19, 1192 (1936); Johnston, J. Am. Chem. Soc., 63, 1043 (1941).
[78] Freudenberg, Plankenhorn, and Boppel, Ber., 71, 2435 (1938).
[79] Traill, J. Soc. Chem. Ind., 53, T337 (1934).
[80] Bock, Ind. Eng. Chem., 29, 985 (1937).

cellulose, that the intermediates are mixtures of the three stages of methylation.[81] On hydrolysis, "dimethylcellulose" usually yields mono-, di-, and trimethylglucose, whereas trimethylcellulose yields practically only 2,3,6-trimethylglucose.[74, 82] This result shows that the hydroxyls in positions one, four, and five of the glucose anhydrides in cellulose are blocked.

The presence among the products of hydrolysis of a small amount of 2,3,4,6-tetramethylglucose [83] is now recognized as being the result of degradation of the cellulose during or prior to methylation. Carefully isolated cotton or ramie cellulose yields no tetramethylglucose provided that air is rigorously excluded during methylation.[84] The importance of the presence or absence of tetramethylglucose with respect to the molecular structure of cellulose will be discussed later.

On acetolysis the corresponding acetylated alkyl glucoses are formed; e.g., triethylcellulose yields 1,4-diacetyl-2,3,6-triethylglucose.[85]

Methylene ethers (acetals) of cellulose have been synthesized by allowing formaldehyde [86] or aliphatic methylene ethers [87] or methylal [88] to react with alkali cellulose. The reaction of formaldehyde with cellulose has its parallel in the action of this aldehyde upon *d*-glucose, which leads to methyleneglucose (Tollens), the mechanism being that of acetal formation. Meunier and Gyot [89] believe that, for steric reasons, it is rather improbable that "methylenation" of cellulose takes place on two neighboring —CHOH groups of glucose residues of one and the same chain, for example, on those in positions two and three, because they are in different planes; it is assumed, rather, that two hydroxyl groups of neighboring chains join in this reaction, possibly according to the following scheme:

$$H-\overset{|}{\underset{|}{C}}-O\ \boxed{H+O+H}\ O-\overset{|}{\underset{|}{C}}-H\ \rightarrow\ H-\overset{|}{\underset{|}{C}}O-CH_2-O\overset{|}{\underset{|}{C}}-H$$
$$\underset{CH_2}{\overset{\|}{}}$$
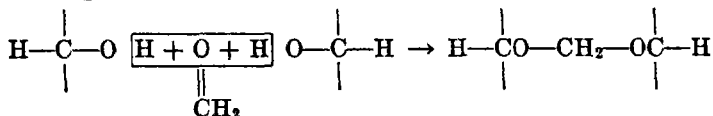
[81] Heddle and Percival, *J. Chem. Soc.*, 249 (1939).

[82] Irvine and Hirst, *J. Chem. Soc.*, **123**, 521, 529 (1923); Hess and Weltzien, *Ann.*, **442**, 46 (1925).

[83] Haworth and Machemer, *J. Chem. Soc.*, 2270 (1932); Haworth and Hirst, *Trans. Faraday Soc.*, **29**, 14 (1933); Averil and Peat, *J. Chem. Soc.*, 1244 (1938); Hirst and Young, *ibid.*, 1247 (1938); Hirst, *J. Textile Inst.*, **27**, 159 P (1936).

[84] Hess, *Angew. Chem.*, **49**, 841 (1936); Hess and Neumann, *Ber.*, **70**, 710, 721, 728 (1937); Leckzyck, *Ber.*, **71**, 829 (1938); Hess and Grigoriscu, *Ber.*, **73**, 499 (1940).

[85] Hess and Wittelsbach, *Z. Electrochem.*, **26**, 244, 250 (1920).

[86] Schenk, *Helv. Chim. Acta*, **15**, 1088 (1932).

[87] Wood, *J. Soc. Chem. Ind.*, **50**, T411 (1931).

[88] Schorigin and Rymaschewskaja, *Cellulosechem.*, **14**, 81 (1933).

[89] Meunier and Gyot, *Compt. rend.*, **188**, 506 (1929).

Glycolic Acid Ether. When monochloroacetic acid is allowed to act upon alkali cellulose, the chlorine reacts with the alkali metal to form sodium chloride. As a result, the glycolic acid ether of cellulose is formed according to the following equation:

$$C_6H_9O_4 \cdot ONa + ClCH_2COOH \rightarrow NaCl + C_6H_9O_4 \cdot O \cdot CH_2COOH$$

This ether forms a sodium salt which in this form is soluble in water, forming a viscous solution. The formulation of the product of reaction as an acid ether of cellulose appears justified, because on the action of phosphorus triiodide and water it liberates glycolic acid ($CH_2OH + CO_2H$) while cellulose is regenerated. A small amount of acetic acid is also formed, which, however, may be due to reduction of part of the glycolic acid.[90]

Triphenylcarbinyl Ether. The formation of this derivative of cellulose may be compared with the action of triphenylchloromethane on alcohols and on certain sugars, such as α-methylglucoside, d-glucose, and d-galactose. The reaction may therefore be formulated thus:

$$C_6H_9O_4ONa + Cl \cdot C(C_6H_5)_3 \rightarrow C_6H_9O_4 \cdot O \cdot C(C_6H_5)_3 + NaCl$$

The ether is very sensitive to acids, e.g., hydrochloric, being cleaved to cellulose and triphenylmethyl chloride (or carbinol), respectively.[91]

Glycolcellulose (Hydroxyethylcellulose). Ethylene oxide reacts with alkali cellulose to form hydroxyethylcellulose (glycolcellulose). It is soluble in water, and it may be assumed that in hydroxyethylcellulose the ethylene oxide radical is present with a free hydroxyl group, $-OCH_2CH_2OH$. The mechanism of the reaction apparently is that of addition and may be illustrated thus:

$$-O-C_6H_7O_2(OH)_2-O- + CH_2-CH_2 \rightarrow$$
$$\underset{O}{\underset{|\_\_\_|}{\quad}}$$

$$-O-C_6H_7O_2(OH)_2(OCH_2CH_2OH)-O-$$

Acetylation leads to a triacetate in which two acetyls occupy the two free hydroxyls of the glucose unit; the third acetyl has entered the hydroxyethylene radical. It may be formulated thus:[92]

$$C_6H_7O_2(OCOCH_3)_2(OCH_2CH_2OCOCH_3)$$

[90] Chowdhury, *Biochem. Z.*, **148**, 85 (1924); see, also, Barnett, *J. Soc. Chem. Ind.*, **40**, T253 (1921); Brown and Houghton, *Chemistry & Industry*, 254 (1941).

[91] Helferich and Koester, *Ber.*, **57**, 587 (1924); Helferich, Moog, and Jünger, *Ber.*, **58**, 872 (1925).

[92] Schorigin and Rymaschewskaja, *Ber.*, **66**, 1014 (1933); Schorger and Shoemaker *Ind. Eng. Chem.*, **29**, 114 (1936).

Benzylcellulose. This cellulose derivative is obtained by the action of benzyl chloride on cellulose in the presence of aqueous alkali. The benzyl ether is exceedingly hydrophobic.[93]

Besides the ethers described above there are a number of others, not yet sufficiently investigated, of which, however, may be mentioned butyl-, propyl-, and allylcellulose.[94]

THE OXIDATION OF CELLULOSE

From an inspection of the molecular structure of the chain molecule, it would appear that with a suitable oxidizing agent the oxidation of cellulose could be confined to an attack which would convert the terminal free reducing groups to carboxylic groups. Furthermore, we could imagine the free hydroxyl groups to be converted to aldehydic and then to carboxylic groups without simultaneous rupture of the glycosidic linkages. However, as pointed out in the introduction, it is difficult to direct the oxidation of cellulose in such a way, and only a few examples in which glycosidic linkages seem to remain unattacked are known.

As with other cellulose reactions the course and the rate of oxidation are essentially influenced by the peculiarities of the fibrous structure. As a result, oxidation proceeds in topochemical fashion, but the products of the initial reaction are further attacked and broken down to shorter chains before deeper layers of the fiber are attacked. Thus, after a certain time has elapsed, the product of reaction, "oxycellulose," must be expected to be a heterogeneous mixture of more or less oxidized and broken chains with secondary reaction products of rather low molecular weight.

As a matter of fact, oxycellulose shows both aldehyde and acid reactions. When heated in dilute alkali it develops a characteristic yellow color, the coloring matter dissolving in the hot alkali. Oxycellulose possesses pronounced reducing power toward Fehling's solution or Willstätter and Schudel's hypoiodite solution;[95] it is capable of reducing vat dyes to their leuco bases,[96] and it reacts with phenyl-

[93] Gonfard, "Sur les propriétés de la benzylcellulose," Camus, Lyon (1933); Georgi and Lorand, *J. Am. Chem. Soc.*, **59**, 1166 (1937).

[94] Mienes, "Celluloseester und Celluloseäther unter besonderer Berücksichtigung der Benzylcellulose," Chem.-techn. Verl. Bodenbender, Berlin (1934); Marsh and Wood, "An Introduction to the Chemistry of Cellulose," p. 273. For dibasic acid esters, see Malm and Fordyce, *Ind. Eng. Chem.*, **32**, 405 (1940).

[95] Willstätter and Schudel, *Ber.*, **51**, 780 (1918).

[96] Scholl, *Ber.*, **44**, 1312 (1911); Ermen, *J. Soc. Dyers Colourists*, **28**, 132 (1912); Haller, *Helv. Chim. Acta*, **14**, 578 (1931).

hydrazine.[97] Its acid nature is indicated by its affinity for basic dyes, such as methylene blue, by its consumption of alkali on titration,[98] and by the liberation of carbon dioxide on boiling with dilute hydrochloric acid.[99] The simultaneous liberation of furfural indicates the presence of glucuronic acid units. The quantities of these two substances as well as the amount of alkali consumed on titration, although certainly greater than from untreated native cellulose, are rather small, which indicates that the number of carboxyl groups in oxycellulose is somewhat limited. Obviously, most of them are oxidized further; in fact, a considerable amount of carbon dioxide is liberated during oxidation.[100]

Whether the aldehydic or acidic character prevails in oxycellulose depends upon the oxidant and the medium, as well as on the time allowed for the reaction. Oxycelluloses of the reducing type are generally obtained with oxidants which may be used in an acid or neutral medium, whereas alkaline oxidation produces the non-reducing or acidic type. In both cases, the aldehydic character prevails at the beginning of the reaction.[101]

Oxycellulose may be freed of most of its aldehydic and acidic character by extraction with dilute alkali. On such a treatment, which involves not only extraction of the fragments of low molecular weight but also oxidation of aldehydic and neutralization of carboxylic groups, the far greater part, remaining in the form of a residue, does not seem to differ from the original cellulose. Yet, determination of its viscosity in solution reveals that it possesses a much lower degree of polymerization than the original fiber, indicating that longer chains have suffered cleavage into smaller fragments. The residue may be further fractionated into portions of varying degree of depolymerization.[102]

[97] Müller, Helv. Chim. Acta, 22, 208, 217, 376 (1939).

[98] Schwalbe and Becker, Ber., 54, 545 (1921); Hibbert and Parsons, J. Soc. Chem. Ind., 44, T473 (1925); Karrer and Lieser, Cellulosechem., 7, 1 (1926); Lüdtke, Biochem. Z., 268, 372 (1934); 285, 78, 82 (1936); Neale and Stringfellow, Trans. Faraday Soc., 33, 881 (1937); Sookne and Harris, Textile Research, 19, 405 (1940); J. Research Natl. Bur. Standards, 26, 205 (1941); Sookne, Fugitt, and Steinhardt, ibid., 25, 61 (1940); Heymann and Rabinow, J. Phys. Chem., 45, 1152, 1167 (1941).

[99] Heuser and Stöckigt, Cellulosechem., 3, 61 (1922); Hibbert and Parsons, loc. cit.; Whistler, Martin, and Harris, J. Research Natl. Bur. Standards, 24, 13 (1940); Nickerson and Leape, Ind. Eng. Chem., 33, 83 (1941).

[100] Cunningham and Dorée, J. Chem. Soc., 101, 497 (1912); Dorée and Healey, J. Textile Inst., 29, T27, T41, (1938).

[101] Clibbens and Geake, J. Textile Inst., 15, T27 (1924); Birtwell, Clibbens, and Geake, ibid., 17, T145 (1926); Clibbens and Ridge, ibid., 18, T135 (1927); Clibbens, Geake, and Ridge, ibid., 18, T277 (1927); Birtwell, Clibbens, Geake, and Ridge, ibid., 21, T87 (1930); Davidson, ibid., 23, T95 (1932); Neale, Trans. Faraday Soc., 29, 228 (1933). See also the compilation by Wise, Trans. Electrochem. Soc., 73, 79 (1938).

[102] Goldman, Haworth, and Peat, J. Chem. Soc., 1908 (1939).

The products of lower molecular weight, those of oligo- and monosaccharide character, are difficult to separate. Among them, glucuronic acid has been identified. Earlier investigators, such as Tollens and Vignon, had suggested that oxycellulose might be defined as polyglucuronic acid. The results of later studies [103] seem to support this view. When a cuprammonium solution of cellulose is oxidized with potassium permanganate, the resulting glucuronic acid seems to retain a polymeric character until, for the purpose of isolation, it is converted into the cinchonine salt of the monomeric acid.

Glucuronic acid (and polyglucuronic acid) long escaped discovery, probably because on oxidation of cellulose in fibrous form it is easily destroyed. Oxidation of cellulose in solution permits a more homogeneous reaction and increases the chances of arresting oxidation before the products of reaction are further attacked and converted into fragments of low molecular weight, such as oxalic acid, and finally into carbon dioxide and water.

An interesting reaction has been observed to occur when periodic acid acts upon cellulose. [104] With this oxidant the attack is confined to the hydroxyl groups in the 2- and 3-positions of the glucose residues. These groups are converted into carbonyl groups, but, apparently, no glycosidic linkages are broken. As a result a cellulose dialdehyde is obtained.
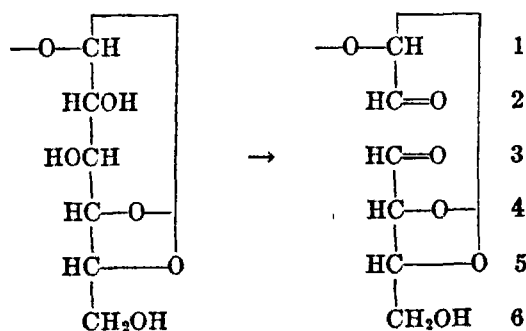
However, under the influence of weak alkali, the dialdehyde breaks down into smaller fragments. [105]

Oxycellulose is formed during a number of commercial processes such as kier boiling and bleaching of textiles, paper pulp, and the like. Overbleaching may lead to a tendering of the fiber. On further oxidative

[103] Kalb and Falkenhausen, Ber., 60, 2514 (1927); see, also, Heuser and Stöckigt, Cellulosechem., 3, 61 (1922); Kenyon et al., J. Am. Chem. Soc., 64, 121, 127 (1942).

[104] Jackson and Hudson, J. Am. Chem. Soc., 59, 2049 (1937).

[105] Davidson, J. Textile Inst., 29, T215 (1938); Dorée and Healey, ibid., 29, T27 (1938)

treatment the cellulose fiber becomes so brittle that it may be disintegrated to powder by mere rubbing between the fingers.

THE DEGRADATION OF CELLULOSE BY ACIDS

With mineral acids, and less completely with organic acids, hydrolysis leads to glucose through a number of intermediate stages; on acetolysis, the hydroxyls of the degradation products become partly or entirely acetylated. Here again, as with other treatments, cellulose reacts slowly and irregularly. Degradation starts on the surface of the fiber and may lead comparatively easily to low-molecular-weight fragments and even to the end product, glucose. Deeper layers, on the other hand, are attacked to a lesser degree, and, in consequence, interruption of the process after a certain time yields a variety of degradation products, from almost untouched chains down to the monomeric glucose.

Hydrocellulose. On heating cellulose fiber with dilute acids, for example with hydrochloric acid, a product is obtained which in appearance and behavior resembles oxycellulose. Like the latter, cellulose emerges from the treatment as weakened fiber which may easily be rubbed to powder between the fingers. It is termed "hydrocellulose" and for many years has been regarded as the first homogeneous product of hydrolysis.[106] The term is derived from the (erroneous) assumption that one molecule of water is chemically attached to each glucose residue. Considering cellulose as a chain of glucose anhydrides linked together by means of oxygen bridges, there is no reason to regard hydrocellulose as a homogeneous cellulose derivative as may be true of oxycellulose. In the formation of hydrocellulose the only change conceivable is a shortening of the chains besides a rupture of cross linkages if such exist. Under the hydrolyzing effect of the acid there is obtained a mixture of chain bundles or single chains, more or less shortened, some of them to oligosaccharides, cellobiose, and glucose, all of which possess pronounced reducing power.

This concept of hydrocellulose [107] is in agreement with its behavior with dilute alkalies. On heating with 4 to 6 per cent sodium hydroxide solution the raw hydrocellulose preparation may be divided into two components. The filtrate shows pronounced reducing power since it contains the lower degradation products. The residue, after sufficient

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[106] See, for example, Schwalbe, "Chemie der Cellulose," Borntraeger, Berlin (1911); see, also, Heuser and Stöckigt, *Cellulosechem.*, **3**, 61 (1922); Heuser and Jayme, *Ber.*, **56**, 1342 (1923).

[107] Heuser, "Lehrbuch der Cellulosechemie," 3rd ed., p. 157; see, also, Heuser and Hinner. *Z. Elektrochem.*, **32**, 47 (1926); Hess, "Die Chemie der Cellulose," p. 439.

purification, shows little, if any, reducing power, probably because the reducing groups which remained after the extraction have become oxidized to carboxylic groups. If the hydrolytic treatment was sufficiently severe, the residue is soluble in sodium hydroxide solution of a certain higher concentration (preferably 8 per cent by weight). The solubility is explained as resulting from the rupture of primary valences; this is also reflected in the solution viscosity of the residue which is considerably decreased in comparison with that of the starting material.[108] The purified alkali-soluble hydrocellulose has also been termed "cellulose A."[109]

It is possible to convert up to 99 per cent of the original cellulose into alkali-soluble hydrocellulose, i.e., with the production of only a very small amount of extractable products of degradation. This result seems to indicate that, as on oxidation, cleavage occurs towards the center of the chains rather than from the ends. Organic acids such as formic,[110] acetic,[111] and oxalic[112] may also be used for the conversion.

Hydrocellulose, which in commercial processes plays a part similar to oxycellulose because the formation of both involves destruction of the fiber, may be distinguished from oxycellulose by certain reactions. Of these may be mentioned the liberation of carbon dioxide on distillation of oxycellulose with 12 per cent hydrochloric acid. Either hydrocellulose does not give this reaction or the amount of carbon dioxide liberated is much smaller than from oxycellulose.[113]

With concentrated acids, such as sulfuric, nitric, hydrochloric, hydrofluoric, or phosphoric, cellulose swells considerably, becomes peptized, and finally dissolves completely. It is likely that intermediate addition compounds are formed.[114] Usually, shortly after preparation, the solution shows reducing power toward Fehling's and other such solutions. On dilution with water shortly after dissolving and with thorough cooling, the greater part of the cellulose may be regenerated in the form of white flakes which show the behavior and the x-ray diagram of mercerized cellulose (p. 1672). In the older literature the preparation obtained is often also termed "amyloid," merely because it gives the same blue coloration with iodine in the presence of traces of

[108] Haworth et al., J. Chem. Soc., 1901, 1904 (1939).

[109] Hess, Weltzien, and Messmer, Ann., 435, 127 (1924); Hess, Z. angew. Chem., 37, 993 (1924).

[110] Heuser and Schott, Cellulosechem., 6, 10 (1925); Staudinger and Dreher, Ber., 69, 1733 (1936).

[111] Heuser, "Lehrbuch der Cellulosechemie," 3rd ed., p. 171.

[112] Heuser and Eisenring, Cellulosechem., 4, 13, 25 (1923).

[113] Heuser and Stöckigt, ibid., 3, 61 (1922).

[114] af Ekenstam, Ber., 69, 549, 553 (1936).

sulfuric acid as starch (amylum) does, a reaction which, although known for more than a century, is not yet fully understood.

Cellodextrins. On further standing of cellulose solutions such as mentioned above, degradation continues, but on the addition of alcohol, a precipitate is obtained. This precipitate, which is characterized by its high reducing power, is partly or entirely soluble in water and has, under the name of "cellulose dextrin" or "cellodextrin," played a great part in the endeavors to isolate homogeneous intermediates in the course of the degradation of cellulose. In the light of the modern concept of the chemical constitution of cellulose, cellulose dextrin is far from being a homogeneous product. It is rather to be regarded as a mixture of oligosaccharides of varying chain length, the longest of which may comprise thirty or less glucose anhydrides.[115] Since the molecules of smaller size crystallize, the whole mass appears crystalline.[116] Likewise certain biose anhydrides, which at one time were thought to represent homogeneous degradation intermediates,[117] are merely mixtures of oligosaccharides of various chain length.

Oligosaccharides. Isolation of certain oligosaccharides is best accomplished by acetolysis or by hydrolysis preceded by methylation or by methylation after acetolysis. The products of reaction are more stable in these cases, and their separation is thus facilitated. Since on acetolysis the hydroxyl groups steadily increase in number and are simultaneously acetylated, because of the progressive opening of oxygen bridges, the increase in acetyl content provides a general means of pursuing the process of degradation.[44] The following oligosaccharides have been isolated: cellohexaose, cellotetraose, cellotriose, and the disaccharide, cellobiose. A mixture of them may be obtained by hydrolysis of cellulose with highly concentrated hydrochloric acid (specific gravity 1.21 at 15°), but the process must be interrupted before it has gone too far. This is accomplished by adding ethyl alcohol, which is used also for fractionating the mixture.[118]

Even cellohexaose, $C_6H_{11}O_6 \cdot [C_6H_{10}O_5]_4 \cdot C_6H_{11}O_5$, the highest member so far isolated, crystallizes in very fine but homogeneous needles; its molecular weight corresponds to the formula given above, and, although it has not yet been possible to obtain crystalline derivatives of this hexasaccharide, derivatives of some of the other oligosaccharides, cellotetraose, $C_6H_{11}O_6 \cdot [C_6H_{10}O_5]_2 \cdot C_6H_{11}O_5$, and cellotriose, $C_6H_{11}O_6 \cdot$

[115] Meyer and Mark, *Ber.*, **61**, 2432 (1928).

[116] Freudenberg, *Ber.*, **62**, 383 (1929).

[117] Bergmann and Knehe, *Ann.*, **445**, 1 (1925); Hess and Friese, *Ann.*, **450**, 40 (1926).

[118] Zechmeister and co-workers, *Ber.*, **64**, 857 (1931); **66**, 269 (1933); see, also, Willstätter and Zechmeister, *Ber.*, **62**, 722 (1929); Zechmeister and Tóth, *Ber.*, **68**, 2134 (1935); Hess and Dziengel, *Ber.*, **68**, 1596 (1935).

$C_6H_{10}O_5 \cdot C_6H_{11}O_5$, are known. These oligosaccharides yield well-defined acetates, methyl ethers,[119] and osazones; the last-named, however, are difficult to obtain in crystalline form. A cellotrioside, decamethyl-β-methylcellotrioside, has been synthesized from 2,3,6-trimethyl-β-methylglucoside and heptamethyl-1-chlorocellobiose.[120]

The mixture of the reaction products also yields cellobiose $(C_{12}H_{22}O_{11})$ (p. 1598), as well as the last link of the chain, d-glucose. For a long time cellobiose was not discovered among the products of ordinary hydrolysis with mineral acids. It is more easily obtained on acetolysis in the form of its octaacetate,[121] $C_{12}H_{14}O_{11}(COCH_3)_8$ with some pentaacetyl-d-glucose as a by-product.[122, 123] On saponification with alcoholic potassium or sodium hydroxide, potassium or sodium cellobiosate is obtained from which cellobiose may be liberated by the action of acetic acid. Another method of saponification, by which the disaccharide constituent is more protected against degradation, consists of applying only traces of sodium metal in methyl alcohol.[124]

Cellobiose may also be obtained by the action of acetyl bromide and glacial acetic acid upon cellulose at a temperature of 30–40° for a number of days. This treatment results in acetobromocellobiose [125] which is identical with E. Fischer and Zemplén's product resulting from the action of hydrogen bromide on cellobiose octaacetate.[126]

Cellobiose, for a long time the only crystalline product of cellulose degradation besides glucose, has played an important part in the attempt to elucidate the chemical constitution of cellulose. Haworth's work [127] indicates its structure to be 1-glucosido-4-glucose with a β-glucosidic linkage.[128]
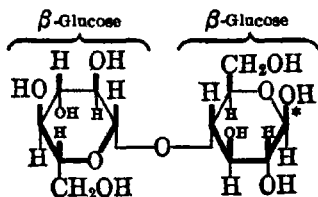
[119] Freudenberg and co-workers, *Naturwissenschaften*, **18**, 1114 (1930); *Ann.*, **494**, 41 (1932).
[120] Freudenberg and Nagai, *Ann.*, **494**, 63 (1932).
[121] Skraup and König, *Ber.*, **34**, 1115 (1901); *Monatsh.*, **21**, 1011 (1900).
[122] Ost, *Ann.*, **398**, 313 (1913).
[123] Webber, Staud, and Gray, *J. Am. Chem. Soc.*, **52**, 1542 (1930).
[124] Zemplén, *Ber.*, **59**, 1254 (1926); **69**, 1827 (1936).
[125] Karrer and Widmer, *Helv. Chim. Acta*, **4**, 700 (1921).
[126] Fischer and Zemplén, *Ber.*, **43**, 2536 (1910).
[127] Haworth, "The Constitution of Sugars," Arnold and Co., London (1929).
[128] See also Zemplén, *Ber.*, **59**, 1254 (1926); Freudenberg, *et al.*, *Ber.*, **63**, 1962 (1930)
* The free reducing group is indicated by an asterisk in the unit on the right.

"Isocellobiose," which was thought to be formed simultaneously with cellobiose on hydrolysis or acetolysis of cellulose,[129] is now recognized as a mixture of cellobiose with oligosaccharides.[130]

Cellobiose may be transformed into a great number of derivatives. Detailed discussions and descriptions of more recent date may be found elsewhere.[131]

The end product of hydrolysis of cellulose is d-glucose. The theoretical yield of glucose is 111.1 per cent by weight. Actually this yield has never been obtained because of inversion[132] as well as of further degradation of glucose to organic acids, hydroxymethylfurfural, and other substances, under the influence of the hydrolyzing agents applied.[133] The best yield may be obtained by allowing methyl alcoholic hydrochloric acid to act upon cellulose triacetate; by this method 95 per cent of the theoretical yield is obtained.[134]

The action of hydrogen bromide in ether on cellulose in a sealed tube leads to about 33 per cent of ω-bromomethylfurfural. This result has for some time been interpreted to indicate the presence of ketonic groups in cellulose.[135] The primary product of this reaction, however, is glucose, which subsequently loses water and is transformed into hydroxymethylfurfural and further into the bromo derivative.[136] In fact, hydroxymethylfurfural may be obtained directly from aldoses as easily as from ketoses.[137]

Anhydrous hydrogen chloride seems to convert cellulose into a mixture of polymeric glucose anhydrides ("polyglucosans"). This conversion seems to involve two steps, namely: (1) cleavage of the chains into glucosylchloride (1-chloroglucose) molecules, and (2) condensation of these molecules with the loss of hydrogen chloride to polyglucosan. On treatment with dilute mineral acid the polyglucosan is transformed into glucose.[138] Hydrogen fluoride acts similarly, the reaction product

[129] Ost and Prosiegel, Z. angew. Chem., **33**, 100 (1920); Ost and Knoth, Cellulosechem., **3**, 25 (1922); Ost, Z. angew. Chem., **39**, 1117 (1926); Hess, Weltzien, and Singer, Ann., **443**, 71 (1925).

[130] Freudenberg, "Tannin, Cellulose, Lignin," Springer, Berlin (1933), p. 99.

[131] See Tollens-Elsner, "Kurzes Handbuch der Kohlenhydrate," Barth, Leipzig (1935), p. 436; Micheel, "Chemie der Zucker und Polysaccharide," Akad. Verlags-Ges., Leipzig (1939).

[132] Schlubach and Lührs, Ann., **547**, 73 (1941); Frahm, Ber., **74**, 622 (1941).

[133] Ost and Wilkening, Chem. Ztg., **34**, 461 (1910); Monier-Williams, J. Chem. Soc., **808** (1921).

[134] Irvine and Hirst, J. Chem. Soc., **121**, 1585 (1922); Heuser and Aiyer, Z. angew. Chem., **37**, 27 (1924).

[135] Fenton and Gostling, J. Chem. Soc., **79**, 361, 807 (1901).

[136] Heuser, "Lehrbuch der Cellulosechemie," 3rd ed., p. 211.

[137] Heuser and Eisenring, Cellulosechem., **4**, 20 (1923); Heuser and Schott, ibid., **4**, 85 (1923); **6**, 10 (1925); Middentrop, doctoral dissertation, Leiden (1917).

[138] Schlubach and Prochownick, Angew. Chem., **47**, 132 (1934); see, also, Hess and Ulmann, Ber., **74**, 119 (1941) and Ulmann and Hess, Ber., **74**, 136 (1941).

of anhydride character being termed "cellan." It is probable that a labile glucosyl fluoride is formed as an intermediate.[139]

THE DEGRADATION OF CELLULOSE BY THERMAL DECOMPOSITION

The products of thermal decomposition, in contrast to those of hydrolysis and acetolysis, yield no information in regard to the chemical constitution of cellulose. These products represent the result not only of far-reaching degradation, but also of secondary, tertiary, and further reactions, so that it is often difficult to explain their presence.

If a temperature of about 270° is applied, as is usual in commercial destructive distillation of wood, a great quantity of gas is produced, consisting chiefly of carbon dioxide and, particularly at higher temperatures, of carbon monoxide, with smaller amounts of methane and ethylene. The process of destructive distillation of cellulose may be regarded as a carbonization with elimination of water and carbon dioxide, whereas carbon monoxide and all other products owe their formation to secondary, tertiary, and further reactions. The principal other products are tar and acetic acid, together with some formic acid and acetone, the last two being formed long before the temperature of 270° has been reached.

Methyl alcohol, which before its synthetic manufacture was an important product of commercial distillation of wood, owes its formation chiefly to the lignin constituent of the wood.[140] It is obtained from cellulose only by the process of hydrogenation in the presence of catalysts.[141]

The tar produced on destructive distillation of cellulose consists chiefly of phenols, which indicates that transformation of aliphatic into aromatic substances has occurred under the influence of the high temperature.[142] The composition of the solid residue, cellulose coke, deviates somewhat from that of anthracite, its hydrogen content being slightly lower. However, if cellulose is subjected to heating under a pressure of 150 atmospheres, in the presence of water (in order to avoid overheating), the composition of the resulting coke approaches that of anthracite.[143]

Destructive distillation of cellulose in vacuo yields levoglucosan or

[139] Helferich and Peters, Ann., 494, 101 (1932); Fredenhagen and Cadenbach, Angew. Chem., 46, 113 (1933).

[140] Büttner and Wislicenus, J. prakt. Chem., 79, 177 (1909); Heuser and Skiöldebrand, Z. angew. Chem., 32, 41 (1919), Heuser and Schmelz, Cellulosechem., 1, 49 (1920).

[141] Fierz-David and Hanning, Helv. Chim. Acta, 8, 900 (1925); Chemistry & Industry, 44, 942 (1925).

[142] Smith and Howard, J. Am. Chem. Soc., 59, 234 (1937).

[143] Bergius, Naturwissenschaften, 16, 1 (1928); Berl, Papier-Fabr., 31, 141 (1933); see also, Heuser, Z. angew. Chem., 26, 393 (1913).

β-glucosan (yield about 38 per cent), i.e., a glucose anhydride [144] (p. 1622). It is identical with that which may be obtained on hydrolysis of glucosides, e.g., from picein, which splits into piceol (*p*-hydroxyacetophenone) and levoglucosan under the influence of strong bases. Structurally, *β*-glucosan is 1,6-anhydro-*β*-*d*-glucopyranose. Its *β*-configuration is indicated by the fact that it is obtained easily from *β*-*d*-glucose and *β*-*d*-glucosides but not from *α*-*d*-glucose or *α*-*d*-glucosides.[145] It is also obtained on destructive distillation *in vacuo* from glucose directly, as well as from starch.[146] These observations seem to indicate that the primary product of reaction is glucose and that glucose subsequently undergoes dehydration resulting in the formation of the anhydride.

Levoglucosan adds one molecule of chloral to form chloralglucose ("chloralose").[147] An analogous reaction seems to occur with cellulose when subjected to treatment with chloral in the presence of concentrated sulfuric acid, various dichloralglucoses being formed.[148] Again, it is likely that glucose is the primary product of reaction, for glucose is converted into chloralglucose directly when subjected to the action of chloral in the presence of sulfuric acid.

THE DEGRADATION OF CELLULOSE BY MEANS OF BIOLOGICAL PROCESSES

Cellulose plays an important part in many biological processes. Nature has provided for permanent destruction of cellulose waste by the activity of microörganisms, such as aerobic and anaerobic, mesophilic and thermophilic bacteria, as well as fungi, actinomyces, and protozoans. Little is known about the mechanism of the breakdown of cellulose by the activity of these microörganisms, but it is generally assumed that the breakdown occurs in two phases, the first consisting of enzymatic hydrolysis which leads to glucose as an intermediate and the second being the fermentation of the glucose to organic acids and gases.

It has been possible to demonstrate the production of glucose by introducing certain antiseptics which are capable of checking the

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[144] Pictet and Sarasin, *Helv. Chim. Acta*, 1, 87 (1918); Venn, *J. Textile Inst.*, 15, T414 (1924).

[146] See Tollens-Elsner, "Kurzes Handbuch der Kohlenhydrate," Barth, Leipzig (1935), p. 515.

[146] Zemplén and Gerecs, *Ber.*, 64, 1545 (1931).

[147] Pictet and Reichel, *Helv. Chim. Acta*, 6, 621 (1923); White and Hixon, *J. Am. Chem. Soc.*, 55, 2438 (1933); W. Freudenberg and Vajda, *ibid.*, 59, 1955 (1937).

[148] Ross and Payne, *J. Am. Chem. Soc.*, 45, 2363 (1923).

fermentative action of the microörganisms without inhibiting the enzymatic hydrolysis.[149] Moreover, Pringsheim was able to show also that cellobiose appears as an intermediate by raising the temperature to a point at which the enzyme which breaks down cellulose to glucose (cellulase) becomes inactive while the cellobiose-producing enzyme (cellobiase) is still active. Under these conditions only cellobiose is formed.

The ability of cellulose-decomposing enzymes to exert specific actions was further demonstrated by Grassmann and co-workers[150] with cellulase and cellobiase containing extracts obtained from *Aspergillus orycae*. It was found that cleavage of longer chains (from cellulose down to cellodextrin and further to cellohexaose) is effected exclusively by cellulase whereas the other oligosaccharides including cellobiose undergo scission chiefly under the action of cellobiase.

The acids produced on unchecked fermentation are chiefly acetic and butyric. Sometimes alcohols, and usually gases, such as methane, hydrogen, and particularly carbon dioxide, are produced, depending chiefly upon the nature of the organism concerned.[151] In recent years, industrial utilization of cellulose waste has come into prominence.[152]

THE CHEMICAL CONSTITUTION OF CELLULOSE

In the chapter published in the first edition of this book an attempt was made to depict the development of the concept of the constitution of cellulose. Here it may suffice to summarize the more essential scientific events which justify our present concept of its molecular structure.

On the basis of the knowledge that cellulose on hydrolysis yields essentially glucose and, on acetolysis, an appreciable amount of di- or

[149] van Iterson, *Zentralbl. Bakt.* II, 11, 689 (1904); Pringsheim, "Die Polysaccharide," Springer, Berlin (1931), p. 152.

[150] Grassmann, Zechmeister, Tóth, and Stadler, *Ann.*, 503, 167 (1933); Grassmann, Stadler, and Bender, *Ann.*, 502, 20 (1933).

[151] For more detailed information see Thaysen and Bunker, "The Microbiology of Cellulose, Hemicelluloses, etc.," Oxford University Press (1927), and Waksman, "Principles of Soil Microbiology," Williams and Wilkins Co., Baltimore (1932); Waksman and Davidson, "Enzymes," Williams and Wilkins Co., Baltimore (1926), as well as the following publications: Symons and Buswell, *J. Am. Chem. Soc.*, 55, 2028 (1933); Waksman and Cordon, *Soil Sci.*, 45, 199 (1938); Walker and Warren, *Biochem. J.*, 32, 31 (1938); Gray, *Can. J. Research*, C17, 154 (1939); Baker, *Nature*, 143, 522 (1939); Berl and Koerber, *J. Am. Chem. Soc.*, 60, 1596 (1938). See also Norman, "The Biochemistry of Cellulose, etc.," Clarendon Press, Oxford (1937).

[152] Fontaine, Peterson, and Ritter, paper presented before the Cellulose Division of the American Chemical Society at Cincinnati, Ohio, April, 1940; Prescott and Dunn, "Industrial Microbiology," McGraw-Hill Book Co., New York (1940).

trisaccharide,[152] Tollens [154] proposed a formula for cellulose (1895) which shows a number of glucose anhydride units linked in chain fashion by way of oxygen bridges (Fig. 2). It will be noticed that the individual

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                                         O
                        H₂ H  H  H  H   ‖
                        C - C - C - C - C - C
                           │   │   OH OH OH H
                           O   O
                     H₂ H  H  H  H  
                     C - C - C - C - C - C
                        │   │   OH OH OH H
                        O   O
               H₂ H  H  H  H  
               C - C - C - C - C - C
                  │   │   OH OH OH H
                  O   O
         H₂ H  H  H  H  
         C - C - C - C - C - C
         OH OH OH OH OH H
```
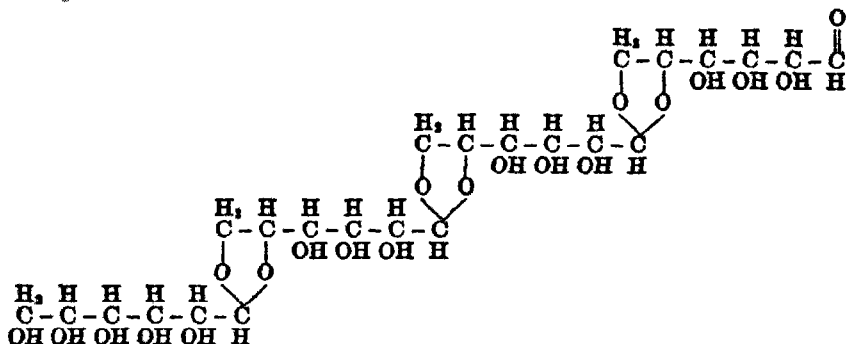
Fig. 2.—Cellulose formula (Tollens).

glucose residues are united through acetal linkages in contrast to the hemi-acetal linkage operative in the modern concept of the molecular structure of the cellulose chain. Tollens believed that such a double oxygen linkage would best explain the great physical resistance of the cellulose fiber.

If for the moment we set aside the attempts of Hess and his school and other investigators to show that cellulose represents an aggregate of monomeric glucose anhydrides united through other than primary valence forces, the generation after Tollens occupied itself chiefly with endeavors to add such experimental evidence as would support the concept of a chain formula.

The essential experimental facts brought to light during the last forty years may be summarized as follows:

1. Esterification and etherification lead to derivatives in which not more than three hydroxyl groups are substituted. This fact was established for cellulose acetate by Ost [155] and for methylcellulose by Denham and Woodhouse.[156]

2. Hydrolysis of trimethylcellulose yields essentially trimethylglucose whose structure is recognized as being a 2,3,6-trimethylmonose.[156, 157, 158]

[152] Franchimont, Ber., 12, 1941 (1879); Skraup and Hamburger, Ber., 32, 2413 (1899); Franchimont, Rec. trav. chim., 18, 472 (1899).

[154] Tollens, "Handbuch der Kohlenhydrate," Trevendt, Breslau (1895), Vol. II, p. 252.

[155] Ost, Angew. Chem., 32, 66, 76, 82 (1919).

[156] Denham and Woodhouse, J. Chem. Soc., 103, 1735 (1913); 105, 2357 (1914); 111, 244 (1917); Denham, ibid., 119, 81 (1921).

[157] Haworth and Leitch, J. Chem. Soc., 113, 191 (1918).

[158] Irvine and Hirst, ibid., 123, 518 (1923); Hess and Weltzien, Ann., 442, 46 (1925).

3. The yield of 2,3,6-trimethylglucose amounts to more than 90 per cent.[158]

These three facts, together with the results of the hydrolysis of cellulose itself, make it appear very probable that cellulose consists of glucose units only and that in each of them the hydroxyl groups in the 1- and 4-positions are occupied. With cellobiose known as one of the intermediates of acetolysis (Skraup, 1901) there is little doubt that one hydroxyl, probably that in the 4-position of one glucose unit, is glycosidically linked with the other glucose unit of cellobiose. Likewise, it is probable that in this second unit (the non-reducing component of cellobiose) the hydroxyl group in the 4-position is connected by the same type of linkage with a third glucose unit.
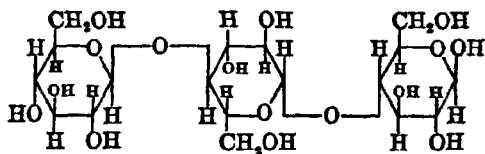
4. In the light of the statistical probability that a chain consisting of glucose or cellobiose residues must yield considerably less than 100 per cent cellobiose, and considering the fact that losses due to further cleavage of the cellobiose formed and to other causes are considerable, the maximum yield of 43 per cent actually obtained (Madsen, 1917) is quite in agreement with the molecular chain concept. [159]

5. The structure of cellobiose is that of 1-glucosido-4-glucose [160] (p. 1697).

6. Glucose possesses an amylene oxide ring between the carbon atoms in the 1- and 5-position [161] (p. 1556).

7. Hydrolysis of cellulose with strong hydrochloric acid yields, besides glucose and cellobiose, the oligosaccharides cellotriose and cellotetraose; [162] from the products of acetolysis cellotriose may be isolated [163] and in a comparatively high yield if acetolysis is arrested before cellobiose is formed.[164]

8. To the products of acetolysis, one more oligosaccharide has been added, namely, cellohexaose.[165] The glucose residues in the oligosac-

[158] Freudenberg, Ber., 54, 767 (1921).

[160] Charlton, Haworth, and Peat, J. Chem. Soc., 89 (1926); Zemplén, Ber., 59, 1254 (1926); Freudenberg, Ber., 63, 1962 (1930).

[161] Hirst, J. Chem. Soc., 350 (1926).

[162] Willstätter and Zechmeister, Ber., 62, 723 (1929).

[163] Bertrand and Benoit, Compt. rend., 177, 85 (1923); 176, 1583 (1923); Ost, Z. angew. Chem., 39, 1117 (1926).

[164] Irvine and Robertson, J. Chem. Soc., 1488 (1926).

[165] Zechmeister and Tóth, Ber., 64, 857 (1931); Zechmeister, Mark, and Tóth, Ber., 66, 269 (1933); Staudinger and Leupold, Ber., 67, 479 (1934).

charides are found to be united through the same type of linkage that is operative in cellobiose.

9. That the cellobiose linkage is not restricted to one part of the cellulose molecule is also apparent from the results of the methylation of cellodextrin which, as has been seen, consists of a number of oligosaccharides. Hydrolysis of the mixture of the methylated products yields tetramethylglucose and a completely methylated cellotriose, which may be broken down to octamethylcellobiose (after further methylation of the degradation products) and a one-third proportion of tetramethylglucose. Thus, four contiguous units originating from cellulose are found to be united in the same manner as cellobiose itself.[166]

10. The discovery of a small quantity of 2,3,4,6-tetramethylglucose besides a large quantity of 2,3,6-trimethylglucose among the products of hydrolysis of trimethylcellulose (Haworth and Machemer, 1932) indicates the existence of long chains, one of their terminating units having a fourth hydroxyl group in the 4-position.

11. Derivatives of cellobiose and cellotriose may be obtained by allowing 2,3,6-trimethyl-β-methylglucoside to react with the chlorohydrins of fully methylated glucose (tetramethylglucose-1-chlorohydrin) and fully methylated cellobiose (heptamethylcellobiose chlorohydrin). In these derivatives, because of the fact that they were synthesized from components in which the mode of linkage is known, the glucose units are joined by β-glycosidic linkages. It is probable, therefore, that the same mode of linkage is operative also in the other oligosaccharides. This assumption is supported by the results of the quantitative evaluation of the optical superposition of the oligosaccharides. Hence, it is very probable that in cellulose also all linkages belong to the β-series. One α-linkage, like that in maltose, to one hundred β-linkages, would reveal itself by a perceptible change in molecular rotation.[167]

Further support of the assumption that only one type of linkage is operative between the individual glucose units is derived from the kinetics of hydrolysis and acetolysis of cellulose. As these reactions proceed, more and more carbonyl groups are exposed, the number of which may be quantitatively estimated. After the fundamentals of such investigations had been established by Meyer, Hopff, and Mark,[168] and by Kuhn,[169] the numerous investigations of Freudenberg and

[166] Haworth, Hirst, and Thomas, J. Chem. Soc., 824 (1931).

[167] Freudenberg and Nagai, Ann., 494, 63 (1932); Freudenberg and co-workers, Ber., 63, 1961 (1930).

[168] Meyer, Hopff, and Mark, Ber., 62, 1103 (1929); 63, 1531 (1930).

[169] Kuhn, Ber., 63, 1503 (1930).

others[170] during recent years leave no doubt that the linkages in cellulose are all of the β-type.

Thus, at the present time, cellulose is regarded as an aggregate of chains in which a large number of glucose units are linked together in a mode corresponding to that which occurs in cellobiose, namely, through 1,4-β-glycosidic linkages. On the assumption that the chains are open, there are two terminating units of which the one possesses a free hydroxyl in the 4-position and the other a free reducing group in the 1-position (see Fig. 1).

The Molecular Weight of Cellulose. The (hypothetical) molecular weight of an individual chain equals the number of glucose units multiplied by the molecular weight of the unit. Since cellulose is visualized as an association of chains of varying length, the molecular weight of the cellulose substance is composed of the molecular weights of all chains which participate in this association.

In the formula of the molecular structure of a cellulose chain (see Fig. 1) each of the two end groups in the two terminating units represents a certain percentage of the total chain. Since the ratio of either end group to normal groups changes with the chain length, the assessment of the total quantity of one of the end groups in some form, in percentage of a given quantity of cellulose, should supply a measure of its average chain length.

A method which has been frequently used for this purpose was suggested by Haworth and Machemer.[171] Essentially it consists of separating the products of hydrolysis of fully methylated cellulose. As illustrated in Fig. 3, a given quantity of cellulose will yield a certain

Hydrolysis gives tetramethylglucopyranose.   Hydrolysis gives 2, 3, 6-trimethylglucose and MeOH.
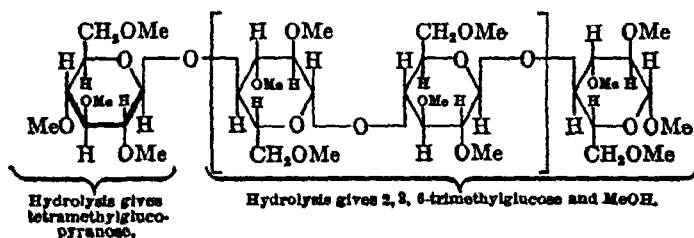
FIG. 3.—From Haworth, "The Constitution of Sugars," Arnold and Co., London (1929). (Courtesy of the publishers.)

[170] Freudenberg et al., Ber., **63**, 1510 (1930); Freudenberg, "Tannin, Cellulose, Lignin," p. 99 et seq.; Freudenberg and Blomqvist, Ber., **68**, 2070 (1935); Trans. Faraday Soc., **32**, 75 (1936); Blomqvist, Sitzber. heidelberg. Akad. Wiss. Math. naturw. Klasse, **7** (1936); Freudenberg, Monatsh., **69**, 144 (1936); Mark and Simha, Trans. Faraday Soc., **36**, 611 (1940); Montroll and Simha, J. Chem. Phys., **8**, 721 (1940); Sakurada and Okamura, Z. physik. Chem., A187, 289 (1940); Simha, J. Applied Phys., **12**, 569 (1941).

[171] Haworth and Machemer, J. Chem. Soc., 2270 (1932); Haworth and Hirst, Trans. Faraday Soc., **29**, 14 (1933); Haworth, Monatsh., **69**, 314 (1936).

quantity of 2,3,4,6-tetramethylglucose whereas the rest will give 2,3,6-trimethylglucose. Basing their calculation on a yield of tetramethylglucose of 0.6 per cent Haworth and Machemer arrived at an average chain length of their cellulosic material of not less than 100 and not more than 200 glucose units which would correspond to a molecular weight between 20,000 and 40,000.

It should be kept in mind that the value thus obtained cannot be generalized because it represents the chain length of the specific cellulosic material used. In the case under discussion the starting material was acetone-soluble cellulose acetate which, as has been seen, contains cellulose in a considerably degraded state. As mentioned before, cellulose carefully isolated from cotton and ramie and methylated with rigid exclusion of atmospheric oxygen yields no tetramethylglucose. This result may be explained by the assumption that in (practically) undegraded cellulose the chains are not open but united with each other through their terminating units or else they are so long that the very small quantity of tetramethylglucose cannot be detected by analytical methods.

Besides, even where greater quantities of tetramethylglucose may be expected, the reservation must be made that the cumbersome operations of separating the methyl sugars and of purifying the tetramethylglucose involves certain losses which, in view of the relatively very low yield of tetramethyl sugar, might result in considerable error. This error increases with increasing chain length of the starting cellulose material. Even under most rigid precautions the values obtained will yield only an approximate idea of the chain length.

Other end group determination methods, namely, that of Bergmann and Machemer,[172] which consists of determining the aldehyde groups of the cellulose material, and that of Schmidt,[173] which is based upon the assumption that native cellulose possesses terminal carboxylic groups, must be valued with even greater reservation.

In recent studies of the methylation of cellulose Haworth and co-workers [174] made the observation that on methylation with the exclusion of air (in a nitrogen atmosphere) and subsequent hydrolysis of the methylated cellulose no tetramethylglucose could be obtained. On the

[172] Bergmann and Machemer, *Ber.*, **63**, 316, 2304 (1930); Staudinger and Schweitzer, *Ber.*, **63**, 3132 (1930); Staudinger, "Die hochmolekularen organischen Verbindungen"; Ulmann, "Molekulgrössen-Bestimmungen hochpolymerer Naturstoffe," Steinkopff, Dresden (1936), p. 100. Bergmann and Machemer's method has recently been improved by Martin, Smith, Whistler, and Harris. *Am. Dyestuff Reptr.*, **30**, 628 (1941).

[173] Schmidt et al., *Ber.*, **69**, 366 (1936); see, also, Sookne and Harris, *J. Research Natl. Bur. Standards*, **25**, 47 (1940); Rebek, *Kolloid-Z.*, **92**, 217 (1940).

[174] Haworth, Hirst, Owen, Peat, and Averill, *J. Chem. Soc.*, 1885 (1939); Haworth, Montonna, and Peat, *ibid.*, 1899 (1939).

other hand, the molecular weight of the trimethylcellulose as determined, for example, by the osmotic pressure method (see below) gave proof of the fact that the chains had been shortened as a result of the conditions of methylation. This paradox is explained on the assumption that the end groups which must have been formed on the breakdown of the chains have become involved in some form of recombination so that no end groups remain. Since on methylation in air subsequent hydrolysis does reveal the presence of end groups, air obviously operates to inhibit this recombination. It remains to be seen whether this interpretation and the conclusions drawn therefrom as to the structure of cellulose can be further substantiated.[175]

In recent years renewed efforts have been made to overcome the difficulties which are encountered when the classical physical methods of molecular-weight determination are applied to high-polymeric substances. It now appears that the osmotic-pressure method may be applied to cellulose derivatives in solution with a considerable measure of reliability.[176] Moreover, methods have been developed which permit the conversion of cellulose into its derivatives without degradation so that extrapolation from the derivative to cellulose itself rests on a safer basis than before.

Another physical method is based upon the relationship between the solution viscosity of cellulose and its degree of degradation. According to Staudinger [177] the viscosity of cellulose in cuprammonium solution or of cellulose derivatives in organic solvents increases over a wide range proportionally with increasing chain length, and vice versa. This postulate, which represents a modification of Einstein's relationship between the viscosity of a substance in solution and its average (spherical) particle volume, implies that in solution, provided that the concentration is low enough, the chain molecules of cellulose exist as kinetically and chemically independent units ("macro- or thread molecules").

In order to calculate the molecular weight the viscosity is used in connection with a constant according to the following equation

$$\frac{\eta_{sp}}{C_{gm}} = K_m \cdot M$$

in which M is the molecular weight; η_{sp}, the specific viscosity, i.e., the

[175] Haworth, *Chemistry & Industry*, 917 (1939); see, also, Bawn, Hirst, and Young, *Trans. Faraday Soc.*, **36**, 880 (1940), referring to starch.

[176] Dobry, *Kolloid-Z.*, **81**, 190 (1937); Wo. Ostwald, *ibid.*, **81**, 195 (1937); Meyer and Wolff, *ibid.*, **89**, 196 (1939); Montonna and Zilk, *J. Phys. Chem.*, **45**, 1374 (1941).

[177] Staudinger and Heuer, *Ber.*, **63**, 222 (1930); Staudinger, *Z. physik. Chem.*, **153**, 391 (1931); *Ber.*, **65**, 267 (1932); Staudinger, "Die hochmolekularen organischen Verbindungen," pp. 56 and 483.

viscosity which a dissolved substance produces in the solvent; and C_{gm}, the concentration of the solution in basic moles per liter. If the value of the constant K_m is known, the molecular weight of a given cellulose material may be calculated simply from viscosity measurements. In order to establish the K_m value, the molecular weight of cellulose or its derivative has to be determined in some way, preferably by the osmotic-pressure method. From both the molecular weight and the viscosity data the K_m value may be calculated, since $K_m = \eta_{sp}/CM$. The K_m constant is different for different cellulose derivatives and for cellulose itself.

The reliability of the viscosity method for calculating the molecular weight depends upon a variety of prerequisites. These, however, are not always fulfilled, and the results should be used with the understanding that the method permits only a relative assessment of the molecular weight. Staudinger and co-workers usually express the results as "degree of polymerization" (D.P.) which is obtained by dividing the molecular weight, as found, by the weight of the single glucose anhydride unit (or derivative unit).

Numerous determinations have been carried out according to this method. Staudinger reports good agreement between molecular weights obtained with the viscosity method and those resulting from osmotic-pressure measurements. On the basis of more recent determinations [178] molecular weights of 324,000 to 480,000 are given for native (cotton) cellulose, of 38,000 to 112,000 for cellulose triacetate, and of 54,000 to 410,000 and higher for nitrocelluloses.

The hypothesis that in proper solution cellulose or cellulose derivatives are separated into their individual chain molecules has in recent years been the subject of some controversy. Increasing difficulties in defending it arise from the possibility that the individual chains are connected by cross linkages of primary valency nature. If such cross linkages are maintained on dissolving and during the conversion of cellulose into derivatives, the viscosity of the solution must be related to the unseparated associations of chain molecules. This would have an essential bearing upon the justification of using viscosity measurements as a means for determining chain length and upon the habit of identifying chain length with molecular weight.

The ultracentrifugal method,[179] originated by Svedberg and first

[178] Staudinger and Schulz, *Ber.*, **68**, 2320 (1935); Schulz, *Z. physik. Chem.*, **176**, 323 (1936); Staudinger and Daumiller, *Ann.*, **529**, 251 (1937); Staudinger and Mohr, *Ber.*, **70**, 2296 (1937).

[179] Stamm, "Colloidal Chemistry of Cellulosic Materials," *U. S. Dept. Agr. Bull.*, *Misc. Pub.*, 240, Washington, D. C. (1936), p. 29; Kraemer and Lansing, *J. Phys. Chem.*, **39**, 153 (1935); Lansing and Kraemer, *J. Am. Chem. Soc.*, **57**, 1369 (1935); Kraemer, *Ind. Eng. Chem.*, **30**, 1200 (1938).

applied to proteins, utilizes photographic observations and records of equilibrium or velocity sedimentation in a strong centrifugal field (up to 150,000 revolutions and more per minute). The values found for cellulose and cellulose derivatives generally are higher than those obtained with the other methods. The following figures are given: 570,000 for native cellulose (corresponding to 3600 glucose units); 150,000 to 500,000 for purified cellulose; 50,000 to 120,000 for cellulose regenerated from viscose, etc.; and 45,000 to 100,000 for cellulose acetates. Kraemer and Lansing believe that the values obtained represent the molecular weights of the cellulose preparations under investigation with as great a certainty as those obtainable by the classical methods applied to substances of low molecular weight.

By using ultracentrifugal data, Kraemer [180] has established a constant which may be used instead of Staudinger's K_m constant for calculating the molecular weight of cellulose from viscosity measurements. The values thus obtained are higher by 10–15 per cent than those obtained by using Staudinger's constant, the difference being explained on the assumption that the osmotic-pressure method gives "number"-average molecular weights whereas the ultracentrifugal method yields "weight"-average values.[181]

THE FINE STRUCTURE OF CELLULOSE AS REVEALED BY X-RAY ANALYSIS

As mentioned earlier in this chapter (p. 1665), x-ray analysis furnishes definite proof of the crystalline nature of cellulose. It appears that most of the investigators ascribe the lattice on which the cellulose crystal is built to the monoclinic system, with dimensions of the basic cell, i.e., the smallest unit which still possesses the geometrical properties of the whole crystal lattice, expressed in Å ($1 \text{ Å} = 10^{-8} \text{cm.} = 0.1 \text{ m}\mu$), as follows (Meyer-Mark-Andress values): [182]

a	(horizontal)	8.35
b	(vertical, representing the length of the basic cell, parallel to the fiber axis)	10.3
c	(forming the angle with a)	7.9
$\beta = 84°$		

These dimensions being known, the volume of the unit cell may be calculated, and, from the volume and the mass of the anhydroglucose formula unit and the density of cellulose, it has been found that four cellobiose residues form the edges of the unit cell, whereas one passes through the center. However, it must be kept in mind that the four cellobiose residues are shared by the neighboring unit cells.

[180] Kraemer, reference 179.
[181] Kraemer and Lansing, reference 179, p. 165.
[182] Mark, "Chemie und Physik der Cellulose," pp. 138, 141.

Making use of the work of W. H. Bragg and his numerous collaborators on the radii of atoms and the distances between atoms of homopolar compounds, Sponsler and Dore [183] devised a picture of the possible structural arrangement of the glucose units in the basic cell.

It is most interesting to note that a decision with regard to the glucose structure to be selected for the arrangement within the unit cell could be made from three-dimensional models carefully constructed to a scale based upon the atomic radii of carbon and oxygen and the distances C—C and C—O. It was found that the amylene oxide ring structure, which, as a result of chemical evidence, had been suggested by the Haworth school, fitted best into Sponsler's lattice spacing as derived from x-ray data. For reasons of symmetry, the β-structure was given preference to the α-structure.

For the mode of linking between neighboring glucose units of the chain, Sponsler and Dore erroneously chose alternating glycosidic and ether linkages instead of glycosidic linkages only. However, this has practically no bearing as far as their conception of the arrangement of the glucose units within the basic cell is concerned. The most essential result was that the investigators recognized the recurrency period (b) of 10.25 Å along the fiber axis, as evidenced by x-ray investigations, to be dependent upon the chemical structure of cellulose. The diameter of one glucose unit, using Haworth's pyranose ring structure, was calculated to be 5.13 Å, that is, just half of the recurrency period, which means that in the unit cell, within the spacing of 10.25 Å on each chain, there occur two glucose units or one cellobiose unit (Fig. 4).* This sug-
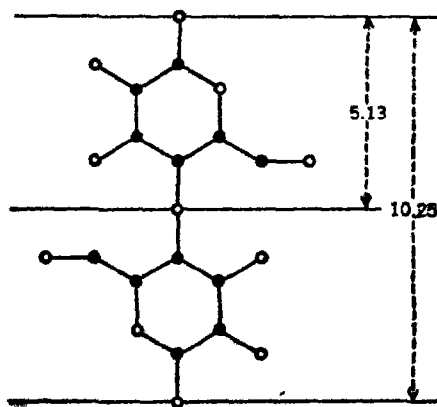
5.13

10.25

Fig. 4.—From Sponsler and Dore, in "Colloid Symposium Monograph IV," Chemical Catalog Co. (1926). (Courtesy of the publishers.)

[183] Sponsler and Dore, "Colloid Symposium Monograph IV," p. 174 (1926); see, also, Sponsler, *Am. J. Botany*, 9, 471 (1922).

* In this and the following figures, the hydrogen atoms are omitted.

gested that the constituent units are arranged in continuous chains, which run parallel to the fiber axis through the unit cell. The position of the chains with respect to each other, stabilized by secondary valency forces, is shown in Fig. 5.
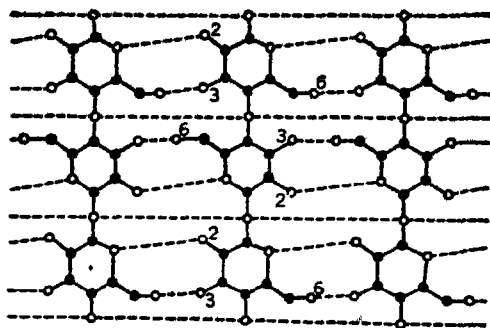
FIG. 5.—From Sponsler and Dore, in "Colloid Symposium Monograph IV," Chemical Catalog Co. (1926). (Courtesy of the publishers.)

Sponsler and Dore's structure would explain a number of the physical phenomena dealt with in previous sections. It would account, for instance, for the anisotropic swelling of the cellulose fiber in water or in other liquids, which do not attack it chemically. Swelling is slight in the longitudinal direction of the chains, since there is apparently no opportunity for the molecules of these liquids to penetrate between the single units of the chain. In the lateral direction, however, molecules of the liquid find sufficient space to enter and in so doing widen the space still further. This theory is in agreement with the x-ray pattern of the swollen (mercerized) cellulose.[184]

Sponsler and Dore's picture would also account for the courses of the chemical reactions which cellulose undergoes. For example, the fact that fibrous structure may be retained on esterification and etherification can be explained by assuming that the new groups insert themselves into the spaces between the longitudinal chains. This will occur more easily the smaller the groups, a concept which is well supported by the work of Trillat[185] and others.

It must be emphasized that Sponsler and Dore's most interesting results gave great impetus to the studies in the years which followed their presentation. Notably, K. H. Meyer,[186] and Meyer and Mark[187]

[184] Andress, Z. physik. Chem., B4, 201 (1929); Katz, in Hess, "Die Chemie der Cellulose," p. 755; also, Trans. Faraday Soc., 29, 279 (1933); Meyer, Angew. Chem., 50, 900 (1937); Hermans, Kolloid-Z., 88, 172 (1939).

[185] Trillat, Compt. rend., 197, 1616 (1933); Trillat and Motz, ibid., 198, 2147 (1934).

[186] Meyer, Z. angew. Chem., 41, 935 (1928).

[187] Meyer and Mark, Ber., 61, 593 (1928); Meyer and Mark, "Der Aufbau der hochpolymeren organischen Naturstoffe," Akad. Verlags-Ges., Leipzig (1930), pp. 93, 113.

devoted much thought and experimental study to the problem. Their endeavors were facilitated by the accumulation of chemical evidence on questions of constitution, particularly the establishment of the cellobiose formula by Haworth and his school and the abundance of x-ray data gathered from the study of numerous organic compounds.

Meyer and Mark followed Sponsler and Dore's procedure, i.e., they constructed three-dimensional models of the constituent units from balls having multiples of the atomic radii and distances that had been established on other compounds. They also gave consideration to the tendency of the carbon atom to arrange neighboring carbon atoms tetrahedrally around itself.
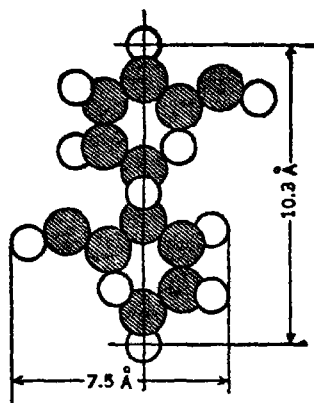
Fig. 6.—From Meyer and Mark, "Aufbau der hochpolymeren organischen Naturstoffe," Akad. Verlags-Ges., Leipzig (1930). (*Courtesy of the publishers.*)

Further aid was derived from Staudinger's ideas on the structure of polymers as well as from investigations by Müller and Shearer,[188] and others of the Bragg school, on long-chain fatty acids. Later, Meyer and Brill[189] obtained support from studies on well-formed crystals of lauric acid in which the molecules were found to lie parallel to each other, and the atoms in the chains were found to be arranged in zigzag form.

In their endeavors to accommodate the glucose units in a basic cell, Meyer and Mark used the dimensions as established in 1921 by Polanyi. Further calculations were based upon the β-form of Haworth's cellobiose model as represented in Fig. 6.* For the construction of this model, 1.54 Å was chosen as the distance between carbon atoms and

[188] Müller and Shearer, *J. Chem. Soc.*, **123**, 3156 (1923); Müller, *Proc. Roy. Soc. (London)*, **A114**, 542 (1927).

[189] Meyer and Brill, *Z. Krist.*, **67**, 570 (1928).

* The shaded circles indicate the carbon atoms; oxygen atoms are represented by the other circles; hydrogen atoms are omitted.

1.35 Å was chosen as the distance between carbon and oxygen atoms.

By turning the lower part of the model through 180° and shifting it upward it will cover exactly the upper part of the model. Thus, the cellobiose configuration reveals the principle of a diagonal screw arrangement. X-ray analysis has shown that the same principle prevails parallel to the fiber axis and that the screw component equals half the recurrency pattern, that is, 5.13 Å.

Since the length of the cellobiose model measures 10.25 Å, it is evident that this length is almost identical with that of the recurrency pattern.
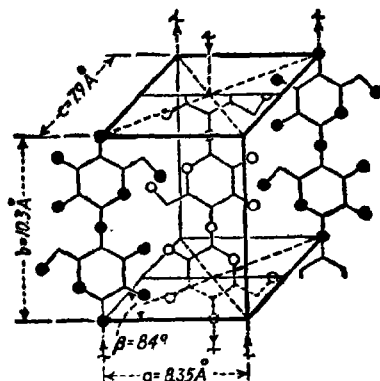
Combining the various pieces of evidence, Meyer and Mark concluded that in the basic cell the cellobiose residues lie parallel to the b-axis. Meyer and Mark's arrangement recently modified in accordance with Meyer and Misch's suggestion,[190] i.e., with the glucose residues pointing alternately in opposite directions, is shown in Fig. 7.

Recently Sauter has proposed a unit cell for cellulose which deviates materially in some dimensions from that suggested by Meyer and Mark. However, the controversy which arose between Meyer and Mark and Sauter seems to be closed by the result of investigations which Gross and Clark [191] undertook with the object of arriving at a definite decision. These authors could show that the x-ray patterns which they obtained from very different types of cellulose were all consistent with the Meyer and Mark unit cell.

[190] Meyer and Misch, *Helv. Chim. Acta*, **20**, 232 (1937).

[191] Gross and Clark, *Z. Krist.*, **99**, 357 (1938); *Textile Research*, **9**, 7 (1938); see, also, Kiessig, *Z. physik. Chem.*, **B43**, 79 (1939).

An approximate idea of the size and the shape of the micellae or crystallites has been derived from the breadth of the hyperbolas (layer lines) of the diagram. Accordingly, the micella of the ramie fiber is calculated to be a rhombus which measures at least 600 Å along the fiber axis and 50 by 50 Å across this direction.[192]

The micellae in the native-cellulose fibers are all oriented parallel to the fiber axis; in cotton they are turned spirally around the axis,[193] whereas in artificial fibers and in films orientation is missing unless it is produced by stretching. The flax fiber shows the highest degree of orientation.[194] The orientation of the micellae is directly related to the strength of the fibers, since the latter increases with the degree of the former.

Meyer and Mark's original conception of the possible arrangement of the micellae in the fiber is shown in Fig. 8. Here (a) indicates primary
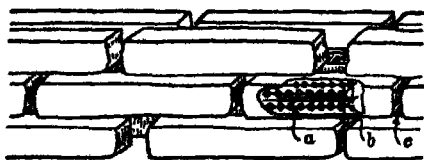
FIG. 8.—From Clark, "Applied X-Rays," McGraw-Hill Book Co., New York (1932), 2nd ed.   (Courtesy of the publishers.)

valences between the glucose anhydrides, (b) secondary forces holding the chains in bundles, and (c) "tertiary" or "micellar" forces between the micellae.[195]

At the present time Meyer and Mark's conception of the micellar structure of cellulose is undergoing some essential changes. These changes appear to be justified, chiefly (1) by the recognition that x-ray analysis reveals a considerable part of the cellulosic substance to be non-crystalline in nature and (2) by the probability that the chain molecules, on the average, are much longer than has been deduced from x-ray data. These considerations have led to an arrangement as depicted in Fig. 9, which represents Frey-Wyssling's conception.[196]

[192] R. O. Herzog, J. Phys. Chem., 30, 457 (1926); Hengstenberg and Mark, Z. Krist., 69, 271 (1928); Clark, Ind. Eng. Chem., 22, 474 (1930).

[193] Farr and Clark, Contrib. Boyce Thompson Inst., 4, 273 (1932); see, also, Steinberger, Textile Research, 4, 495, 531 (1934).

[194] Morey, Textile Research, 4, 491 (1934).

[195] A similar arrangement is shown in Hawley and Wise, "Chemistry of Wood," American Chemical Society Monograph, Chemical Catalog Co., New York (1926), p. 26. The picture seems to originate from Seifriz, Am. Naturalist, 63, 410 (1929).

[196] Frey-Wyssling, Protoplasma, 25, 262 (1936); 26, 45 (1936); 27, 372, 563 (1937); Kolloid-Z., 85, 148 (1938).

Similar arrangements have been suggested by Kratky,[197] by Mark,[198] and others.

With such arrangements the micellae have lost their former individuality. Rather they appear as chain bundles of indefinite length although they have retained their thickness of about 50 by 50 Å. The
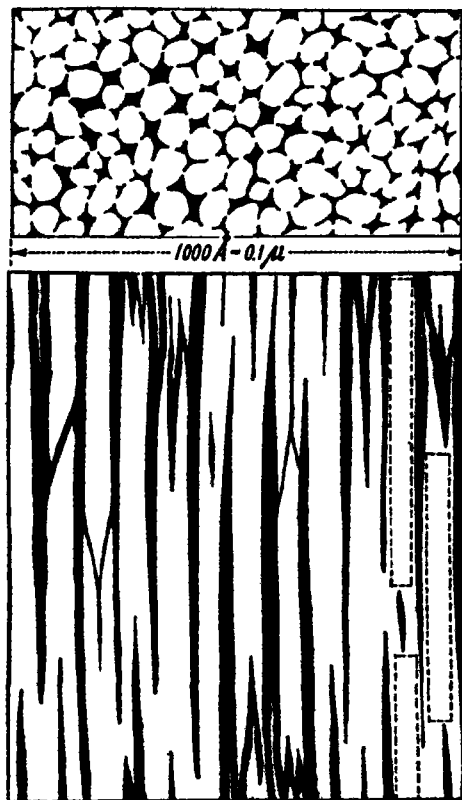
FIG. 9.—Schematic picture of the submicroscopic structure of native bast fibers. Above, transverse section; below, longitudinal view, much reduced in size. The black spots and lines represent the intermicellar spaces. From Frey-Wyssling, *Papier-Fabr.*, **36**, 214 (1938). (Courtesy of the publishers.)

chain bundles are separated by intermicellar spaces of widely varying width. The parallel orientation is thought to be interrupted at irregular intervals. Where this state prevails that portion of the chain bundles is regarded as being of an amorphous or non-crystalline ("unordered")

[197] Kratky, *Naturwissenschaften*, **26**, 94 (1938); *Silk and Rayon*, **13**, 480, 572, 634 (1939).

[198] Mark, *J. Phys. Chem.*, **44**, 764 (1940). This article is an excellent summary of the studies which have led to the modern conception of the micellar structure of cellulose.

nature, whereas the strictly parallel portions (indicated by the dotted lines in the picture) represent crystalline ("ordered") regions. Thus, the chains are assumed to pass through crystalline and amorphous regions. The modern concept is gaining in popularity since it obviously is able to explain the properties and reactions of cellulose more satisfactorily than the older concept of individual micellae of limited longitudinal dimensions.

THE FIBRILLAR STRUCTURE OF CELLULOSE

The cell wall may be dissected lengthwise into fibrillae or fibrils, i.e., thin elongated units, 100 to 230 and more microns long,[199] and the fibrils into fragments which are still discernible under the microscope, i.e., sections which are very much larger than the hypothetical micellae or chain bundles. The fibrillar fragments, known under various terms, vary in shape and size, depending upon the method of dissection. For example, Wiesner's "dermatosomes" measure 0.5 μ in length and 0.3 to 0.5 μ in diameter.[200] Similar dimensions were found by Lüdtke, who, moreover, assumes that the length of the fibrils is determined by regularly spaced transverse elements ("Querelemente"),[201] and the shape and size of the fibrillar sections (dermatosomes) by fine membranes ("skin substance"). The existence of transverse elements and of a skin substance could, however, not be confirmed by the botanists.[202]

Other investigators have termed the fibrillar fragments "fusiform bodies" or "spherical units," [203] or just "cellulose particles." [204] Again, depending upon the treatment applied to the cell wall, the dimensions of the observed particles differ. Thus Farr [204] found the particles to measure 1.5 by 1.1 by 1.0 μ, and Wergin [205] claims to have observed particles of only 0.25 by 0.2 by 0.2 μ.

The fibrillae and fibrillar fragments are assumed by some investigators to be held together by a non-cellulosic substance of colloidal and

[199] Ritter, *Paper Ind.*, **16**, 178 (1934); see, also, Bailey and Brown, *Ind. Eng. Chem.*, **32**, 57 (1940).

[200] Wiesner *et al.*, "Die Rohstoffe des Pflanzenreiches," Engelmann, Dresden (1927), 4th ed., Vol. I, p. 396.

[201] Lüdtke, *Ann.*, **466**, 27 (1928); *Biochem. Z.*, **233**, 1 (1931); *Cellulosechem.*, **13**, 169, 191 (1932); **14**, 1 (1933).

[202] Frey-Wyssling, *Papier-Fabr.*, **36**, 214 (1938).

[203] Ritter and Chidester, *Paper Trade J.*, **87**, 159 (1928); Ritter and Seborg, *Ind. Eng. Chem.*, **22**, 1229 (1930).

[204] Farr and Eckerson, *Contrib. Boyce Thompson Inst.*, **6**, 189, 309 (1934); Farr and Sisson, *ibid.*, **6**, 315 (1934); Farr, *Textile Research*, **6**, 513 (1936); *J. Phys. Chem.*, **42**, 1113 (1938); Barrows, *Contrib. Boyce Thompson Inst.*, **11**, 61 (1939).

[205] Wergin, *Protoplasma*, **35**, 116 (1939).

amorphous nature. For example, Hess and his school[206] (particularly Lüdtke[201]) assume a membrane system ("Haut- oder Fremdsubstanz") to penetrate the fibrillar structure of the fiber, whereas Farr and co-workers[207] claim that the cellulose particles, which constitute the fibrillae, are surrounded by a "cementing material" of pectic nature which serves to hold them together. If the cementing material is removed, which is said to be accomplished by treatment of the fiber with pectin-removing agents, as, for example, aqueous ammonium oxalate solution, but also with dilute or strong hydrochloric acid, the cell wall separates into cellulose particles.

Although the various fibrillar fragments observed are of different dimensions they are regarded by the investigators named above as *fundamental* structural units. Farr derives this conclusion from the observation that the particles into which the cell wall may be disintegrated are found to be preformed in the protoplasm of the interior of the young cotton cell. In the later stages of cell development Farr observes beadlike chains made up of particles joined end to end, these chains uniting further to layers which form the cell membrane.

In contrast to the observations of Lüdtke, Farr, and others, I. W. Bailey cannot find any evidence, either in untreated or in carefully swollen fibers, of discrete entities of cellulose, that is, of fibrillae, dermatosomes, or the like, which may be liberated simply by dissolving a non-cellulose constituent. Such units seem rather to be heterogeneous fragments that are shredded or disrupted from an originally continuous and coherent cellulose matrix. Any discontinuities in the structural pattern of cellulose are confined to the submicroscopic field, i.e., to the realm of micellae or molecular chains.[208]

It appears probable that by the various treatments which preceded the observation or the isolation of the fibrillar fragments the cell wall was chemically affected; this was certainly true where the cell wall was subjected to the action of dilute or strong hydrochloric acid for a certain period of time and sometimes at elevated temperature. Under such treatments, which were preferentially applied by most of the above-mentioned investigators, disintegration into particles was no doubt the result of hydrolysis. As a matter of fact, Kraemer[209] found the molec-

[206] Hess, Trogus, Akim, and Sakurada, *Ber.*, **64**, 408 (1931); Hess, *Naturwissenschaften*, **22**, 472 (1934).

[207] Farr, *Contrib. Boyce Thompson Inst.*, **10**, 71 (1938); **12**, 181 (1941); Compton, *ibid.*, **10**, 57 (1938); *J. Am. Chem. Soc.*, **60**, 1807 (1938); Bisson, *Contrib. Boyce Thompson Inst.*, **10**, 113 (1938).

[208] Bailey and Kerr, *J. Arnold Arboretum*, **16**, 273 (1935); Kerr and Bailey, *ibid.*, **15**, 327 (1934); **16**, 273 (1935); **18**, 261 (1937); Bailey, *Ind. Eng. Chem.*, **30**, 40 (1938).

[209] Kraemer, *Ind. Eng. Chem.*, **30**, 1200 (1938).

ular weight of Farr's particles (determined by the ultracentrifugal method) to be only about 40,000. This is less than one-twelfth of that usually found on raw cotton fiber, which Farr and co-workers used as a source for the isolation of cellulose particles.

Farr's observation that the raw cotton fiber on thorough treatment with aqueous ammonium oxalate solution disintegrates into particles could not be confirmed by other investigators,[210] which makes the existence of a cementing substance as a *fundamental* constituent of the cellulose fiber and the conclusion drawn therefrom rather problematical.

Mark [211] has summarized our present knowledge of the structure of the cellulose fiber in a number of imaginary photomicrographs which show what could be seen if it were possible to achieve magnifications up to a point where 1 Å would equal 1 cm. (1:100 million). The reader is referred to this summary.

The use of the electron microscope [212] seems to offer the possibility of making visible such structural elements of the fiber which cannot be detected under the microscope. A great portion of the system of hollow spaces (capillary system) and of the micellae fall within the resolving power of this new tool (3–4 mµ). The results of recent studies [213] seem to confirm the concept derived from x-ray analysis.

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[210] Heuser and Green, *Ind. Eng. Chem.*, **33**, 868 (1941); see, also, Whistler, Martin, and Harris, *Textile Research*, **10**, 269 (1940); Nickerson and Leape, *Ind. Eng. Chem.*, **33**, 88 (1941); Leger and Larose, *Can. J. Research*, **B19**, 61 (1941).

[211] Reference 198, p. 784.

[212] v. Borries and Ruska, *Z. wiss. Mikroskop.*, **56**, 317 (1939); *Z. Physik.*, **116**, 249 (1940); Martin, *J. Soc. Dyers Colourists*, **55**, 214 (1939).

[213] Ruska, *Kolloid-Z.*, **92**, 276 (1940); Ruska and Kretschmer, *ibid.*, **93**, 163 (1940).

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CHAPTER 23

CONSTITUTION AND PHYSICAL PROPERTIES OF ORGANIC COMPOUNDS

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A. WEISSBERGER

Eastman Kodak Company

CONTENTS

GENERAL INTRODUCTION

No sharp borderline distinguishes physical from chemical properties, and this chapter will consider properties like entropy which might be called chemical. On the other hand, not all physical properties of organic compounds which are dependent on their chemical constitution will be dealt with. Some are treated at length in special chapters of this book, and for the others, in view of the limited space, preference will be given to those properties and relationships which are best understood, and to those which are most important for the practical organic chemist. Aside from purely academic interests, knowledge of the relation between chemical constitution and physical properties may be utilized to investigate the constitution of compounds, to check their identity, and to serve as a guide for the production of new compounds with specific properties.

The term "constitution" refers to the arrangement of the atoms in the molecule but does not have a strictly fixed meaning. After a development which in its later stages was marked by the names of Kolbe, Williamson, Dumas, Laurent, Couper, and Gerhardt, the constitution of an organic compound was, from about 1865, in general considered to be fully described by Kekulé's "dash" formulas, which give account of the atoms and bonds, distinguishing the bonds as single, double, or triple. The importance of the spatial arrangement of the atoms in determining the behavior of organic compounds toward polarized light, and *cis-trans* isomerism, was discovered one to three decades later by van't Hoff, LeBel, Wislicenus, Hantzsch, and Werner. Certain conventions were adopted which made it possible to use Kekulé's dash formulas for the description of the geometrical and spatial arrangement of substituents on doubly bound atoms and about "asymmetric" atoms. At the same time it was realized that various organic compounds could not be adequately represented by a single formula, and the conception of tautomerism arose. A new species of linkage, the coördinate bond, was discovered by Werner, and ionizable and unionizable linkages were distinguished. Eventually the electronic theory of valency of G. N. Lewis afforded an interpretation of the latter two types of linkages in terms of atomic physics. The unionizable linkages, for which the "dash" was more and more reserved, was understood to be due to the sharing of two electrons by two atomic nuclei, the electrons being contributed either by both or, in the coördinate link, by one of the atoms. The ionizable link originates in the complete transfer of one electron from the nuclear forces of its parent atom to another atom; this transfer leaves the donor atom with a positive charge and contributes to the acceptor part of the molecule a negative charge.

The ideas of mesomerism, inductive effect, etc., were next conceived, and most recently the quantum-mechanical treatment of London, Heitler, Pauling, and others, led to the comprehensive theory of resonance (see Chapter 26). The ideal constitutional formula is now considered to be a three-dimensional graph which describes the molecule by giving the locations of the atomic nuclei and the distribution probabilities of the electrons. For many purposes, however, the old Kekulé formulas are sufficient, convenient, and advantageous if used with a proper appreciation of their limitations.

It is difficult to determine the value of the various physical properties for the purpose of studying constitution in its various aspects. The evaluation depends on the accuracy of the experimental data and on the theoretical background of their determination and interpretation.

Ostwald [1] classified physical properties as *additive*, *constitutive*, and *colligative*. *Additive* properties are those which are related only to the kind and number of the atoms and are not influenced by the nature of the chemical linkages in the molecule, e.g., the molecular weight.

Constitutive properties depend not only on the kind and number of the atoms, but also on their arrangement in the molecule; their study sometimes allows conclusions to be drawn about the intramolecular arrangement of atoms. Certain constitutive properties, like refractive index, dipole moment, and optical activity, depend only on the individual molecules; these may be termed *constitutive properties of the first order*. Other constitutive properties, like viscosity, surface tension, and melting point, are connected with the aggregation of the molecules; these may be termed *constitutive properties of the second order*. The constitutive properties of the second order depend primarily on intermolecular relations but they are influenced also by the constitution of the interacting molecules, inasmuch as the constitution determines to a greater or less extent the intermolecular forces. The importance of constitutive properties of the second order as affording clues to the constitution of molecules varies with the classes of compounds and with the information which is wanted. Valuable clues can be obtained, for instance, within a polymeric series, by measurements of viscosity, and the fact that a compound has a certain melting point may exclude certain structures and be compatible with others.

Colligative properties as defined by Ostwald do not depend on the chemical nature of the material but only on the number of particles per unit volume, e.g., the pressure of gases, the depression of freezing points, and the exaltations of the boiling points of solvents by a solute. Accord-

[1] Ostwald, "Lehrbuch der allgemeinen Chemie," 2nd ed., Akad. Verlag, Leipzig (1910), I, 1120.

ing to Ostwald no constitutive factor plays a part in these properties. However, where the number of particles depends on the dissociation and association of compounds, which in turn are related to the constitution, colligative properties may offer clues to the arrangement of atoms in the substrates.

The great importance of the constitutive factor in many physical properties is illustrated by a table of some properties of the isomeric xylenes. Richards [2] has pointed out certain relationships existing between various properties. Thus, additive factors being equal, as in a group of isomers, substances with higher densities and smaller molecular volumes have higher boiling points, higher heats of vaporization, higher refractive indices, higher surface tensions, smaller compressibilities, and smaller coefficients of expansion than substances with lower densities.

TABLE I

COMPARISON OF PHYSICAL PROPERTIES OF ISOMERS

Property	o-Xylene	m-Xylene	p-Xylene
Boiling point, ° C.	144	139	136
Melting point, ° C.	−28	−54	+15
Density	0.8811	0.8658	0.8611
Compressibility $\beta \times 10^6$	61.2	64.8	67.5
Coefficient of expansion $\times 10^6$	973	994	1009
Molecular heat of vaporization (Cal.)	8 75	8.71	8.60
Surface tension (mg./mm.)	3.09	2.96	2.92
Refractive index	1.5078	1.5002	1.4985
Pressure to produce M.P. at 30°	490	2400	4000
Dipole moment	0.58	0.46	0
Dielectric constant	2.553	2.371	2.269

Studies by Schurman and Boord [3] on the effect of the shift of the double bond on the properties of a number of isomeric olefins afford other examples of constitutive influences. In Table II are collected

TABLE II

DERIVATIVES OF n-OCTANE

Structure	Boiling Point (° C./760 mm.)	Density, d_4^{20}	Index of Refraction, n_D^{20}
C—C—C—C—C—C—C—C	125.59	0.70279	1.39760
C—C=C—C—C—C—C—C	124.6-124.9	0.7193	1.4147
C—C—C=C—C—C—C—C	122.7-122.9	0.7181	1.4139
C—C—C—C=C—C—C—C	121.9-122.3	0.7184	1.4140
C=C—C—C—C—C—C—C	120.9-121.2	0.7151	1.4091

[2] Richards, *Trans. Faraday Soc.*, **24**, 111 (1928).
[3] Schurman and Boord, *J. Am. Chem. Soc.*, **55**, 4930 (1933); this paper contains references to earlier work in this series.

data on the monoölefinic derivatives of *n*-octane; the properties of *n*-octane are included as reference points. It is seen that except for 1-octene the boiling points become increasingly lower than the boiling point of *n*-octane as the double bond approaches the center of the molecule. The refractive indices and densities do not vary regularly with the position of the unsaturated linkage.

Table III presents data from which may be seen the effect of branched chains and the position of the double bond on the properties of hexenes.

TABLE III

HEXENES

Structure	ΔB.P.	Δd_4^{20}	Δn_D^{20}
C=C—C—C with C above and C below the third carbon	−9.5	+0.0011	+0.0050
C=C—C—C—C with C below the third carbon	−9.5	+0.0054	+0.0042
C=C—C—C—C with C below the fourth carbon	−6.5	+0 0138	+0.0081
C=C—C—C—C—C	−5.1	+0.0137	+0.0107

In the table, Δ values represent deviations from the properties of the corresponding saturated hydrocarbons.

Other general studies by Midgley and Henne,[4] by van Arkel,[5] by Vogel,[6] by Calingaert and Hladky,[7] and by other authors on the relation between constitutive factors and a variety of physical properties of hydrocarbons, esters, acids, and other compounds have appeared in recent years.

The *intermolecular forces* which govern the constitutive properties of second order are those expressed in the value *a* of the van der Waals

$$\left(p + \frac{a}{v^2}\right)\left(v - b\right) = RT$$

equation, and have been rather well elucidated by the work of London, Debye, and Keesom.

[4] Midgley and Henne, *Ind. Eng. Chem.*, **22**, 542 (1930).
[5] van Arkel, *Rec. trav. Chim.*, **53**, 247 (1934).
[6] Vogel, *J. Chem. Soc.*, **333** (1934).
[7] Calingaert and Hladky, *J. Am. Chem. Soc.*, **58**, 153 (1936).

The most important part of this attracting force is that which was explained by London [a] on a theoretical basis by means of wave-mechanics. This force is universal, and effective between non-polar molecules and atoms, e.g., the noble gases, as well as between polar molecules and ions. The values characteristic for the different atoms which enter into the calculation of this force are the same as enter into the dispersion formula; hence the London forces and the atomic and molecular refractions are interrelated, and both are connected with the polarizability of the atoms and molecules.

Molecules which contain permanent dipoles are also, as explained by Keesom,[9] subject to electrostatic forces. These molecules tend to orient each other in a manner which provides the maximum mutual attraction, for instance, as shown by (−+)(−+). Thermal agitation disturbs this arrangement of lowest potential energy and the intermolecular forces are therefore temperature-dependent. In the position shown by the diagram, the dipoles and therefore the attraction of the molecules will increase by polarization. In the reverse position, in which the molecules repel each other electrostatically, their moments will become smaller by polarization and the mutual repulsion will decrease. Hence the polarization of the dipoles contributes in each position toward the attractive forces, as was shown by Debye.[10]

A specific factor contributing to the attraction of molecules, in which the above-mentioned forces combine in a particularly effective way, is found in the hydrogen bonds [11] (see Chapter 25). Enhanced by resonance, these bonds can provide very important intermolecular attractions of about 6 kcal. per mole.

In addition to these attractive forces, there are repulsive forces operating between all atoms and molecules at small distances, which set up rigid barriers to their closer approach. These forces are frequently termed *steric*. Orientations which hinder the contact of the groups and atoms which otherwise would produce strong intermolecular forces, and the reduction of the surface of the molecules, e.g., in branched hydrocarbons as compared with normal ones, can cause very marked effects.

Temperature and pressure influence most of the physical properties of the second order. For purposes of comparison and evaluation, it is therefore important to determine the physical properties under condi-

[a] (a) London, Z. Physik, 63, 245 (1930); (b) Z. physik. Chem., B11, 222 (1930).

[9] Keesom, Proc. Acad. Sci. Amsterdam, 18, 636, 868 (1916); Physik. Z., 22, 129 (1921).

[10] Debye, Physik. Z., 21, 178 (1920); 22, 302 (1921).

[11] Huggins, J. Org. Chem., 1, 407 (1936); Lassettre, Chem. Rev., 20, 259 (1937); Pauling, "Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y. (1940), Chapter IX; Trans. Faraday Soc., 36, 871–929 (1940).

tions in which these imposed influences can be neglected. This is sometimes achieved by extrapolation to limiting conditions, and sometimes by combining the analysis of measurements of several properties into a function which is independent of the experimental conditions and more suitable for treatment. Furthermore, a study of the van der Waals equation shows that substances at the critical points of pressure, temperature, and volume or at equal fractions of them are in *corresponding states*, and suitable for comparison.

As mentioned above, it is sometimes advantageous, in order to obtain information about the constitution of compounds from their physical properties, to derive from the measured data certain functions like molecular refraction, dipole moment, and parachor. This leads to another classification of physical properties into these derived, *secondary* ones, and into the directly measured *primary* data like melting point, refractive index, and dielectric constant.

It will not be possible in this chapter to discuss at length the experimental and theoretical background of the physical properties under consideration. It will be shown, however, for each property, whether it is directly measured or derived, and if derived, from what directly measured data.

The connection between a physical property and the constitution of compounds is usually established by the breaking up of the numerical values of the physical property into values of the structural units which compose the molecules, of atoms and groups, linkages, and other constitutive elements. This analysis may be achieved by inductive or by deductive methods. In the first case, a number of known compounds is submitted to measurements and the results are analyzed. In the second, fundamental theoretical deliberations lead to an understanding of the experimental data in terms of the constitution of the compounds. In the practical cases where physical properties are correlated with constitution, usually a combination of both methods is used.

The properties which are considered in the following first group are determined by measurements of weight, volume, temperature, surface tension, and viscosity. The second group of properties discussed are those which are determined by the observation of their behavior toward electromagnetic waves and electrons.

With rare exceptions, all these measurements are made without the destruction of the substances investigated. The determinations, however, which are the basis for the thermodynamic properties to be considered in the last section, frequently involve irreversible chemical changes.

MELTING POINTS AND BOILING POINTS

The *melting point*[12] is characteristic for crystals. It is the temperature at which the thermal agitation of the units composing the crystal lattice becomes so great that the lattice collapses. The heat which is absorbed in the transition is the latent heat of fusion. The melting point rises with increasing strength of the intermolecular forces and, therefore, with the closeness of the packing of the atoms or atom groups. It also varies with the type of the crystal lattice. These three factors—intermolecular forces, shape of the molecules, and type of the lattice—are interdependent. Organic molecules in many cases are flexible about the single bonds through torsion. They will assume certain configurations according to the forces impressed upon them, and vice versa, and the crystal lattice depends on the shape of the molecules and on the electric fields. Often more than one crystal form representing an energy minimum is possible: polymorphism. Greater symmetry and rigidity of the molecule increase the amount of energy necessary to produce a disturbance through which the lattice collapses; e.g., the melting point of fumaric acid is higher than that of maleic acid, and the melting point of succinic acid lies between the two. This summary shows not only that constitutive factors have ample chance to influence the melting point, but also that their influence is manifold and complex, so that no general simple rules may be expected.

Franchimont, Eykman, and van der Kam[13] found that the melting points of organic compounds rise when two H-atoms bound to the same C-atom are replaced by an O-atom, or one H-atom is replaced by an OH or NH_2 group, and that the melting points are lowered by replacing H which is bound to oxygen or nitrogen by CH_3. These variations are readily understood on the basis of the above general statements; the melting points rise with the introduction of more polarizable atoms and an increased opportunity for hydrogen bond formation. For the same reason, compounds with normal chains have, in general, higher melting points than the isomers with branched chains; the shape of the former gives more opportunity for H , H attractions, which are a source of intermolecular forces.

On the other hand, branched molecules of high symmetry—globular molecules—may have exceptionally high melting points; thus, hexamethylethane melts at 104° C., and *n*-octane at −56.5° C. Timmermans[14] has drawn attention to this phenomenon and suggested that

[12] Timmermans, *Inst. intern. chim. Solvay*, **4**, 191 (1931).

[13] van der Kam, *Rec. trav. chim.*, **45**, 734 (1926).

[14] Timmermans, *J. chim. phys.*, **35**, 331 (1938).

globular molecules absorb a considerable amount of energy as rotational energy without causing a collapse of the crystal lattice. Below the melting point, such substances in the solid state undergo a transformation which in many respects, e.g., in the specific heat and dielectric constant, makes them resemble liquids.

In many homologous series, the melting points rise with increasing molecular weight, evidently through the greater intermolecular forces acting between the larger molecules. With the initial members of homologous series, the addition of a CH_2 group frequently lowers the melting point, because the interference with the forces between the characteristic groups, like $—CO_2H$ and $—OH$, by the new groups is greater than the gain of intermolecular forces through them. In some series with strong forces between the characteristic groups, like the dicarboxylic acids, this lowering of the melting points with increasing molecular weight persists as a general tendency throughout the series. As the effects of the chains dominate those of the characteristic groups, the melting points of the higher members of a number of homologous series approach a common limit of about 120°.[12, 13, 14] However, the convergence points for quite a number of series differ markedly, indicating the importance of the characteristic groups for the packing and the cohesion in the crystals, even in compounds with long chains.

The change in the melting points of homologous series with the addition of CH_2 is in some cases continuous, e.g., with the aliphatic alcohols, the ketones, and the fatty acid amides. In other series, the fatty acids, the normal paraffins, the dicarboxylic acid diamides, the glycols, the alkylmalonic acids, etc., the phenomenon called alternation or oscillation occurs (Fig. 1).
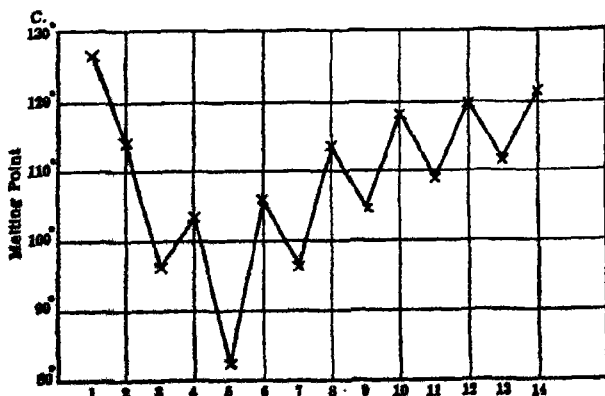
Fig. 1.—Melting points of homologous malonic esters $C_nH_{2n+1}CH(CO_2R)_2$.

Verkade and Coops, Jr., Rec. trav. chim., 49, 565 (1930). (Courtesy of publishers).

[13] Timmermans, II Congr. intern. froid, 1936.
[14] Verkade and Coops, Jr., Rec. trav. chim., 49, 568 (1930).

The oscillation of physical constants, like melting point and heat of crystallization,[17] sometimes consists in the fact that, in a homologous series, with an increase of CH_2, an increase and a decrease of the constant in question alternate (see Figs. 1 and 5); the melting points and the solubility in water of the normal saturated dicarboxylic acids are examples of this type of oscillation. In other cases (see Fig. 2), only
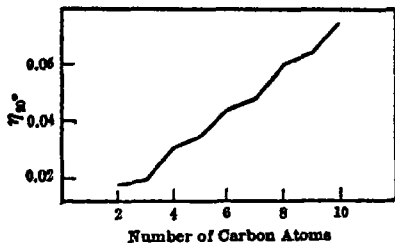
FIG. 2.—Viscosity of diethyl esters of dibasic acids.

From data of Ceder, *Ann. Univ. Fennicae Aboensis*, Ser. A2, No. 4 (1926) [*C. A.*, **22**, 3137 (1928)]. (Courtesy of publishers.)

larger and smaller increases of the constants alternate with each other. No essential difference exists between these two types, which are called *complete* and *incomplete oscillation*. If the decrease or the smaller increase of the constant in question occurs when passing from a member of the series with an even number of carbon atoms to the next (odd) term, the oscillation is called *even* or *normal oscillation;* the reverse case is named *uneven* or *inverse oscillation*.

The oscillation has been interpreted as due to the facts that the hydrocarbon chains have a zigzag form, which was confirmed by x-ray structure analysis, and that the more symmetrical members have a positive increment, the less symmetrical members a negative one. In accordance with this, even oscillation of the melting points is observed with paraffins and terminal dihalogenides, dihydroxides and diamines, the dicarboxylic acids and all their derivatives, while uneven oscillation occurs with alkyl monohalides, mercaptans, etc.

Tammann,[18] on the other hand, explains the alternation by assuming that, for example, fatty acids with even carbon numbers occur in two polymorphic forms, while the odd-membered acids lack the higher-melting modification.

Weygand and Grüntzig[19] contributed an interesting observation to this question. They studied homologous monoacidic triglycerides which show oscillation of melting points, and found that these compounds

[17] Garner, *J. Chem. Soc.*, 2491 (1926).

[18] Tammann, *Z. anorg. allgem. Chem.*, **109**, 221 (1920); **115**, 268 (1921).

[19] Weygand and Grüntzig, *ibid.*, **206**, 313 (1932).

exist in polymorphous modifications. The polymorphous individuals of
the different homologs which closely resemble each other crystal-opti-
cally were arranged in corresponding series, and it was found that the
melting points within these series rise continuously.

A phenomenon of somewhat related nature is the so-called peri-
odicity. The occurrence of anomalous properties in homologous series
at, or in the neighborhood of, the fifth (see for instance Fig. 1), tenth,
and fifteenth members has been interpreted as indicating the arrange-
ment of the carbon atoms in a chain in the form of a spiral or helix.[20]
However, there is no indication of a periodic *repetition* of melting-point
minima. Furthermore, the minima lie, with the fatty acids, at the
compound with a butyl group; with the paraffins and primary aliphatic
alcohols, at the member with the propyl group; with alkyl halides, at
the ethyl halide, and in some series higher than at the five-carbon com-
pound.[16, 20]

In the *benzene* series the melting points rise from the *ortho* to the
meta compounds and from the *meta* to the *para* compounds if the sub-
stituents are of the same kind. Beacall[21] summarizes an investigation
of the melting points of simple benzene derivatives as follows: The in-
troduction of a pair of chlorine or bromine atoms in *para* position to each
other increases the melting point in an approximately constant ratio.

TABLE IV

Introduction of Two Chlorine Atoms in *para* Position

Benzene Compound	M.P. (°K.)	Ratio
Benzene	278.4	
p-Dichlorobenzene	326	1.17
s-Tetrachlorobenzene	410.5	1.26
Hexachlorobenzene	500	1.22
1,2,4-Trichlorobenzene	289.5	
Pentachlorobenzene	358	1.23
Monobromobenzene	242	
1-Bromo-2,5-dichlorobenzene	307	1.27
etc.		

The introduction of a simple "asymmetric" halogen atom into benzene
or asymmetric substituted halogen benzene lowers the melting point in
an approximately constant ratio. The rule of Watt and Carnelley that,
the more symmetrical the constitution of a benzene derivative is, the
higher is the melting point, holds only for derivatives with substituents
of the same kind.

[20] Timmermans, *Bull. soc. chim. Belg.*, **35**, 282, 1126 (1926).

[21] Beacall, *Rec. trav. chim.*, **47**, 37 (1928).

TABLE V

INTRODUCTION OF ONE CHLORINE ATOM

Benzene Compound	M.P. (°K.)	Ratio
Monochlorobenzene	228	
Benzene	278.4	0.82
1,2,4-Trichlorobenzene	289.5	
1,4-Dichlorobenzene	326	0.89
Pentachlorobenzene	358.5	
s-Tetrachlorobenzene	410.5	0.88
1-Bromo-3,4-dichlorobenzene	297.5	
1-Bromo-4-chlorobenzene	339	0.88
etc.		

Formulas have been given from which melting points can be calculated with reasonable accuracy.

Austin [22] has suggested the equation

$$\log M = A + BT_m$$

where M is the molecular weight, T_m is the melting point, and A and B are constants. The constants A and B are generally different for each homologous series, although the constant B, which gives the slope of the curve, may be the same for several different series. Thus, for n-hydrocarbons and n-alcohols, B has the value 0.0040, and hence the $\log M$, T_m curves for these series have the same slope. Variations in the structures of homologous series usually produce changes in the slope of the curves. The curves for monosaccharides, aromatic alcohols, and aromatic acids have negative slopes as contrasted with the positive value of B found for paraffin hydrocarbons.

King and Garner [23] observed a relation between the number of carbon atoms in a fatty acid molecule and the entropy change on crystallization. They found that in acids containing more than twelve carbon atoms the heat of crystallization, Q, increases at the rate of 2.06 kcal. per g.-mol. for every two CH_2 groups added. In the same series, the melting points first drop to a minimum at four or five carbon atoms and then rise and gradually become linear. In this, as in most other aliphatic series, the odd and even carbon compounds form two separate

[22] Austin, *J. Am. Chem. Soc.*, **52**, 1049 (1930).
[23] King and Garner, *J. Chem. Soc.*, 578 (1931).

series, the melting points of which eventually approach a common curve.[34] Figure 3 illustrates the data of King and Garner.
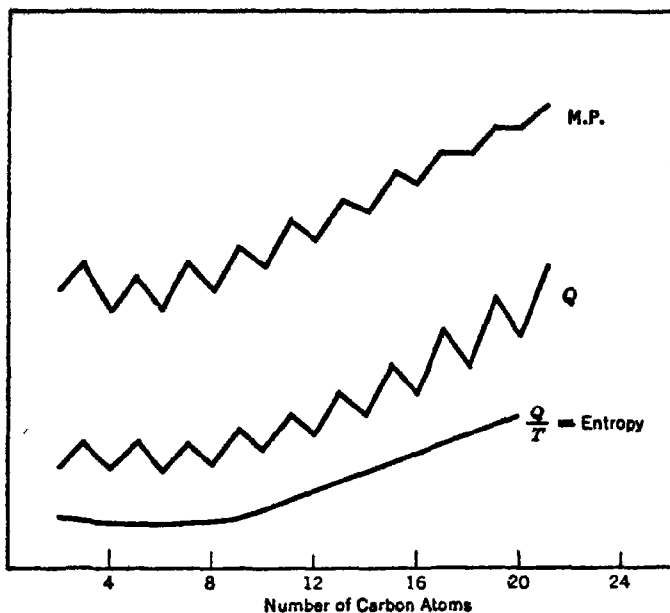
FIG. 3.—Melting points and heat of crystallization of n-fatty acids.

(Q = heat of crystallization in calories per mole)

From data of King and Garner, *J. Chem. Soc.*, 578 (1931).   (Courtesy of publishers.)

The heats of fusion, H, and the melting points in absolute temperature, T, are roughly related by the Crompton-Walden rule:

$$\frac{\Delta H}{T} = \text{constant}$$

At the *boiling point* under atmospheric pressure, the thermal agitation of the particles of a liquid becomes so great that the particles leaving the surface of the liquid exert a pressure of 1 atmosphere.   The energy absorbed in the transition to the gaseous state is the latent heat of vaporization.   The question whether the heat of vaporization is a property measured under conditions suitable for comparison is answered by Guldberg's rule that the boiling point equals about two-thirds of the critical temperature.   Liquids at the boiling point are therefore in corresponding states.

A numerical relation between boiling point in absolute temperature, T_{BP}, and latent heat of vaporization, λ, is given by the Pictet-Trouton rule:

$$\lambda \text{ (in cal.)} = \alpha \, T_{BP}.$$

$\alpha \approx 20$, except for OH-containing substances, where it is about 26. The rule is plausible because both boiling point and heat of vaporization depend on the intermolecular forces.[25] The deviation of the OH-containing compounds may be caused by hydrogen bonds.

Of the numerous equations developed for the prediction of boiling points, the following expression of Nekrasov[26] may be cited as an example:

$$T_{BP} = K \frac{M - \Sigma}{\sqrt{\Sigma}}$$

M is the molecular weight, Σ the sum of certain empirical equivalents given in Table VI, and K a constant (about 29.0 for hydrocarbons).

<div align="center">

TABLE VI

EQUIVALENTS FOR CALCULATION OF THE BOILING POINTS OF ORGANIC COMPOUNDS (Nekrasov)

</div>

C	2.0	Each C atom more than 10	+0.25
H	1.0	=CH$_2$	+0.25
Tert. C in ring	1.50	—CH$_3$ on tert. C	+0.25
Quat. C in ring	1.75	—CH$_3$ on quat. C	+0.50
Double bond in		$\{$=C—C=	−0.50
2 rings	1.00	$\{$=C=	−1.75
Benzene ring	1.00	—CH=CH—	−0.75

Other rings	Sat.	Unsat.		Sat.	Unsat.
3 members	+0.75	......	5 members	0.00	+0.50
4 members	+0.20	+1.00	6 members	0.00	0.00

The calculation of the boiling point of ethane will serve as an example of the use of the data in Table VI. Ethane has 2 carbon atoms and 6 hydrogen atoms; the value of Σ is equal to $2 \times 2 + 6 \times 1 = 10$. If the value 29.0 is used for K, T_{BP} is calculated to be 183°. The boiling point of ethane is observed to be 185° K.

The simpler formula [27] is applicable with non-polar compounds.

$$T_{BP} = \frac{K M^{\frac{2}{3}}}{\Sigma} \qquad \bullet$$

[25] London, Z. physik. Chem., B11, 242 (1930); Hirschfelder and Eyring, J. Phys. Chem., 41, 249 (1937).
[26] Nekrasov, Z. physik. Chem., A141, 378 (1929).
[27] Nekrasov, ibid., A148, 216 (1930).

According to van Arkel,[28] the boiling points of *non-polar compounds* can be computed for molecules containing only carbon and halogen, by means of the formula

$$T_{BP} = \frac{(V - V_c)^2}{V} \cdot K_a$$

V is the molecular volume, V_c the atomic volume of carbon, and K_a is a constant involving the a of van der Waals equation. The square root of a exhibits additive characteristics and can be calculated from atomic values.[8a] Table VII shows comparisons of calculated and observed boiling points. The data of Table VII establish the general

TABLE VII

BOILING POINTS CALCULATED FROM ATOMIC VOLUMES

$V_F = 11.0$ $V_{Cl} = 22.8$ $V_{Br} = 29.1$ $V_I = 39.6$ $V_C = 11$

Compound	V	T_{BP} (calc.)	T_{BP} (observed)
CBr₄	127.4	465°	462°
CClBr₃	121.1	428	433
CCl₂Br₂	114.8	406	408
CCl₃Br	108.5	375	377
CCl₄	102.2	349	349
CCl₃F	90.4	298	298
CCl₂F₂	78.6	249	248
CBr₃F	109.3	380	380
CBr₂F₂	91.2	300	298
CF₄	55	150	143
CCl₃I	119	419	415
CClBr=CClBr	126.8	446	445
CCl₂=CCl₂	114.2	392	394

reliability of the method of calculation for *non-polar* or *only slightly polar* compounds.

More recently, Egloff, Sherman, and Dull[29] have given the equation

$$T = a \log (n + b) + k$$

where T is the boiling point in degrees absolute; n is the number of carbon atoms in the molecule; a, b, and k are empirical constants. When these were evaluated from the boiling points of the *normal alkanes* and the observed boiling points were compared with those calculated according to the formula above, very good agreement was obtained, as shown in Table VIII.

[28] van Arkel, *Rec. trav. chim.*, **51**, 1081 (1932); **52**, 719, 733 (1933); **53**, 91, 246 (1934).
[29] Egloff, Sherman, and Dull, *J. Phys. Chem.*, **44**, 730 (1940).

TABLE VIII

NORMAL ALKANES

$$T = 745.42 \log (n + 4.4) - 416.31$$

Name of Compound	Number of Carbon Atoms	T (Observed) °K.	T (Calculated) °K.	ΔT °K.
(Methane)	(1)	(111.55)	(129.63)	(−18.08)
Ethane	2	184.6	184.6	0.0
Propane	3	230.9	231.6	−0.7
Butane	4	272.6	272.7	−0.1
Pentane	5	309.08	309.08	0.0
Hexane	6	341.88	341.80	+0.08
Heptane	7	371.53	371.52	+0.01
Octane	8	398.88	398.75	+0.13
Nonane	9	423.83	423.85	−0.02
Decane	10	447.1	447.2	−0.1
Undecane	11	468.9	468.9	0.0
Dodecane	12	489.3	489.3	0.0
Tridecane	13	507	508.4	−1.4
Tetradecane	14	524	526.5	−2.5
Pentadecane	15	543.6	543.6	0.0
Hexadecane	16	560.6	559.9	+0.7
Heptadecane	17	576	575.4	+0.6
Octadecane	18	590.0	590.2	−0.2
Nonadecane	19	603 1	604.3	−1.2

The paper contains a similar study of the boiling points of thirty additional analogous series of aliphatic hydrocarbons, the mean deviation between calculated and observed values being only 0.7 per cent for the hundred and forty-three hydrocarbons which were considered. The values a and b were universal for all these series, while k varied from series to series. Generalizations are given on the effect on boiling point of the structures of the hydrocarbons of the different series. For instance, in the straight-chain molecules, a double bond in the terminal position lowers the boiling points (relative to the normal alkanes) by 5° to 6°, a double bond in the 2-position (cis- and trans-2-alkenes) lowers the boiling point less than 0.5°, while the presence of a triple bond in the terminal position raises the boiling point about 2.5°.

When the boiling points of polar compounds are calculated according to van Arkel's formula, there are appreciable differences between the observed and the calculated values. For example, the introduction of bromine into benzene to form bromobenzene gives an observed value

14° higher than that calculated. The discrepancy is due to the polarity of the bromobenzene. Van Arkel has studied the relationships between boiling points and dipole moments in a large number of aromatic and aliphatic compounds and calculated dipole moments from boiling points. Examples of the agreement between observed and calculated moments are found in Table IX.

TABLE IX

DIPOLE MOMENTS CALCULATED FROM BOILING POINTS

Compound	Moment Observed	Moment Calculated	B.P., °C.
o-Dichlorobenzene	2.24×10^{-18}	2.72×10^{-18}	178
m-Dichlorobenzene	1.42	1.57	172
p-Dichlorobenzene	0	0	171 −4
o-Nitrotoluene	3.70	3.74	218
m-Nitrotoluene	4.20	4.15	230
p-Nitrotoluene	4.40	4.38	238

The correlation of the boiling points to the factors governing the intermolecular forces, like dipole moments, polarizability, and presence of hydrogen bonds, has been presented by Hückel,[30] and the following rules will need little explanation, because their connection with the intermolecular forces is evident.

Of isomeric non-cyclic compounds, the one with the normal carbon chain always has the highest boiling point. With increasing branching of the chain, the boiling point falls.

Of isomeric alcohols, halogenides, nitro compounds, etc., the primary compounds have the highest boiling point; the secondary have lower ones; and the tertiary, the lowest ones (screening of the polar group).

Of isomeric bicyclic compounds, those in which the rings are connected by bridges (which give flexibility to the molecule) have lower boiling points than those with condensed ring systems.

Of cis-trans isomers, the cis compound has the higher boiling point and the higher dipole moment.

The bigger and more compact the substituent (screening), the more the approach of a substituent to a carbonyl group depresses the boiling point.

Of isomers with more than one double bond, those with conjugated double bonds have the higher boiling points (higher polarizability of systems of conjugated double bonds than of isolated ones).

A more detailed analysis of the part which the various intermolecular forces play in the boiling points of compounds containing one or two

[30] Hückel, "Theoretische Grundlagen der organischen Chemie," Akademische Verlagsgesellschaft, Leipzig, 1935, Vol. 2, p. 122.

carbon atoms in combination with halogen and hydrogen has been given by Stevels.[31]

SOLUBILITY

Solubility, the temperature coefficient of solubility, and the tendency to crystallize in and from various solvents are very important properties for practical work in organic chemistry. Some generalizations which have been suggested by Hildebrand [32] and others concerning these properties may be useful, although many exceptions can be found. The absence of more reliable relationships between solubility and constitution is understandable, because the solubility depends on intermolecular forces (solvent/solute, solute/solute, solvent/solvent), the connection of which with structural elements is highly complex.

Substances dissolve in water if they can form hydrogen bonds with water (such as alcohols, acids, ketones, ethers, esters, amines, and nitriles).

Non-electrolytes do not dissolve in water if they cannot form hydrogen bonds with water (such as hydrocarbons, halogen derivatives, and carbon disulfide).

The solubility of hydrogen bond liquids in water and in non-hydrogen bond liquids depends on the number of —OH and —NH_2 groups and the size of the hydrocarbon part of the molecule. For instance, methanol is soluble in water but not completely miscible with heptane (at room temperature), while n-butyl alcohol is incompletely miscible with water but completely miscible with heptane.

Relatively high solubilities of halogenated hydrocarbons and of acetylenic compounds in various solvents indicate the existence of CH ← O and CH ← N hydrogen bonds.[33]

Compounds with rigid molecules are less soluble than compounds with flexible molecules.

Of two solids having approximately the same heats of fusion, the one having the higher melting point is less soluble in a given solvent at a given temperature than the one having the lower melting point.

If two solids have equal melting points, the one with the greater heat of fusion will be less soluble in a given solvent.

If two substances have essentially the same heat of solution, their solubilities in a given solvent will be in the order of their melting points.

All normal liquids (non-hydrogen bond liquids) are miscible (unless

[31] Stevels, *Rec. trav. chim.*, **58**, 229, 244 (1939).

[32] Hildebrand, "Solubility," 2nd ed., Reinhold Publishing Corp., New York (1936).

[33] Zellhoefer, Copley, and Marvel, *J. Am. Chem. Soc.*, **60**, 1337 (1938); Copley and Holley, *ibid.*, **61**, 1599 (1939).

their internal pressures are greatly different), since the energy change on mixing is very small and the entropy increases.

A measure of the intermolecular forces mentioned above is given by the internal pressure, i.e., the term a/v^2, in the van der Waals equation, which makes allowance for the deficit in the external pressure. The internal pressure may be approximated by an expression more conveniently measured, l/v, where l is the molecular heat of vaporization and v is the molecular volume.[34] Substances without hydrogen bonds and with internal pressures nearly equal are more soluble in each other than those the internal pressures of which differ appreciably from each other. The differences in solubility of a solute in a series of solvents will, therefore, be determined by the differences between the internal pressures of the solute and the various solvents. Mortimer[34] has tabulated data on the solubility of p-dibromobenzene in a variety of solvents of different internal pressures, and it will be noted that the ideal solubility of 24.8, i.e., the solubility of p-dibromobenzene in pure p-dibromobenzene, is approached when the internal pressure of the solvent is nearly that of p-dibromobenzene. When the internal pressure of the solvent differs greatly from that of p-dibromobenzene, the solubility of the latter is relatively low.

TABLE X

SOLUBILITY OF p-DIBROMOBENZENE

Solvent	Internal Pressure	Solubility, Moles Solute per Liter of Solvent
Hexane	0.56	8.6×10^{-2}
Diethyl ether	0.62	18.3
Carbon tetrachloride	0.81	19 3
Benzene	0.96	21.7
p-Dibromobenzene	1.09	(24 8)
Carbon disulfide	1.18	22.4
Nitrobenzene	1.07	17.4
Aniline	1.4	10.7
Phenol	1.4	4.67
Ethyl Alcohol	2.9	1.98

Hildebrand[35] has pointed out that Raoult's law can sometimes be utilized in the determination of solubilities. Liquid substances showing no heats of solution or no deviations from additivity of volumes on solution in general obey Raoult's law; the evolution of heat or a decrease of volume upon dissolving one substance in another usually indicates a negative deviation from the law, whereas changes in the opposite direction indicate a positive deviation.

[34] Mortimer, ibid., 48, 633 (1923).
[35] Hildebrand, ibid., 51, 66 (1929); 37, 970 (1915).

SURFACE TENSION

Surface tension is the tendency of a liquid to diminish its surface, the term "surface" meaning the interface between the liquid and its vapor or a different gas. It is caused by intermolecular forces and has the dimension dyne/cm. The surface tension between two liquids, called the interfacial tension, is determined by the surface tension of each and by the attractive forces exerted between the unlike molecules.

Many methods have been devised to measure the surface tension, for instance, by determining the weight of droplets, the rise of a liquid in a capillary, or the force necessary to detach a solid from the surface of a liquid.

A general relationship between surface tension and temperature is given by Eötvös,[36] and Ramsay and Shields: [37]

$$\gamma \left(\frac{M}{D-d}\right)^{\frac{2}{3}} = K(T_c - T)$$

where γ is the surface tension, M the molecular weight, D and d the densities of the liquid and gaseous forms of the substances, T_c the critical temperature, T the temperature of observation, and K a constant. K usually has a value of about 2.1 for non-associated liquids when the temperature is expressed in degrees Centigrade.

The equation becomes invalid for associated liquids, in which several molecules of the same species are coalesced to a degree of firmness which enables the group to act as a unit during measurements. Association frequently causes deviations from generalized rules applying to those properties; thus, it is often responsible for boiling points being higher than predicted and for similar increases in viscosity, density, and refractive index. The degree of association is largely determined by the intermolecular forces, of which, in this respect, the most important are the hydrogen bond formations. Therefore, in addition to determinations of molecular weights in solutions, the most significant information on association is obtained from studies of the hydrogen bonds by means of infra-red absorptions and Raman spectra.[38]

One of the various other equations relating temperature and surface tension shows the five-sixths power of the surface tension varying linearly with the temperature. In Fig. 4, where $\gamma^{\frac{5}{6}}$ is plotted against T for several substances, it is seen that many liquids obey the exponential

[36] Eötvös, Ann. Physik, 27, 452 (1886).

[37] Ramsay and Shields, Z. physik. Chem., 12, 433 (1893); see, also, Born and Courant, Physik. Z., 14, 731 (1913).

[38] Rodebush and Buswell, J. Phys. Chem., 43, 219 (1939).

law, whereas certain compounds, such as methyl alcohol and acetic acid, deviate from it. Here, again, the compounds which do not follow the law are associated, and the deviations are probably due to changes in degrees of association with temperature.[39]
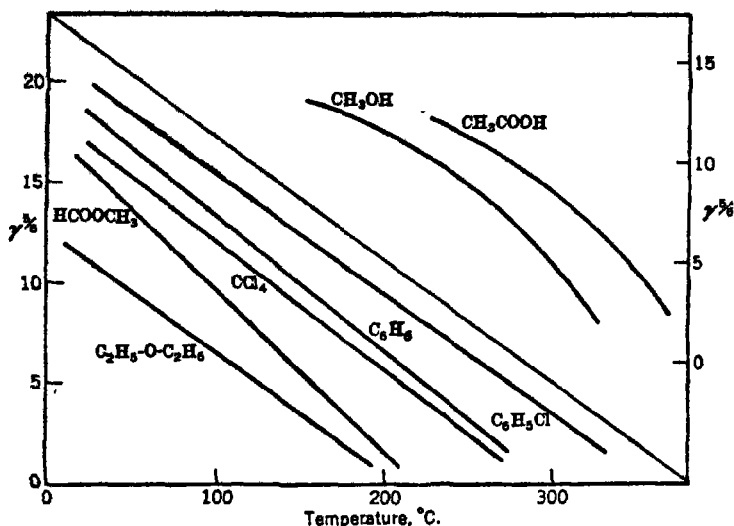
FIG. 4.—Relation between surface tension and temperature.

The analysis of surface-tension data for the purpose of assigning additive and constitutive values for various atomic groups and linkages has not been very successful. This is due, as mentioned above, to the complexity of the effect of these factors on intermolecular forces. It is well known that molecular orientation at the surface plays a large part in the determination of the surface tension of a liquid.[40]

With respect to interfacial tension, Bartell [41] reports that when two liquids are mutually insoluble, e.g., in systems comprising an organic liquid and mercury, the individual interfacial-tension values between one liquid and the members of an homologous series decrease progressively if the surface-tension values of the members of the series increase progressively. With a strongly polar substance like water as one of the interfacial liquids, the interfacial tension of aliphatic compounds decreases in the order: saturated hydrocarbons, unsaturated hydrocarbons, alkyl halides, esters, ethers, ketones, aldehydes, amines, alcohols, acids.

[39] Bartolescu, J. chim. phys., **29**, 418 (1932).
[40] Stewart, Rev. Modern Phys., **2**, 116 (1930).
[41] Bartell and co-workers, J. Am. Chem. Soc., **55**, 2419 (1933).

TABLE XI

INTERFACIAL TENSIONS (Dynes/cm.)

Phase 1	Phase 2		
	Air-Vapor	Water	Hg
Water.	72.75	.....	375
Benzene.	28.86	35.00	364.3
Toluene..	.....	36.1	363.6
Chloroform...	27.1	32.80	357
Aniline...	42.56	5.8	341
Diethyl ether	17	10.7	379
Nitrobenzene....	43.38	25 66	349
n-Hexane..	18.43	51.1	379.9
n-Heptane	.....	. ...	378.7
n-Octane	21.77	50.81	376.0

DENSITY AND RELATED PROPERTIES

The *density* d of a substance is defined by $d = m/v$, where m is the mass and v the volume. Hence, the volume of unit mass is $v = 1/d$, and that of one mole, the molecular volume, $M_v = M/d$, where M is the gram-molecular weight. For ideal gases, d is proportional to M and the molecular volume is constant (22.4 l). The density and, hence, the molecular volume of liquids and solids is a constitutive property, which depends upon temperature and pressure, i.e., on the variation of the intermolecular distances with changes in these two variables. It is therefore important for comparison and evaluation of molecular volumes to determine d at corresponding states, or to reduce the effect of thermal agitation on the intermolecular distances by making measurements at low temperatures or at high pressures.

Biltz [42] and his co-workers have treated the relationships between the *nullpunktsvolume*, obtained by extrapolation of the temperature-density curves to absolute zero, and the constitution of organic compounds in the liquid and solid states. The analysis of the data has led to constitutive and additive numerical constants which give reasonably satisfactory values when compared with those observed. Table XII lists a number of compounds for which the nullpunktsvolumes have been

[42] Biltz and co-workers, Z. *physik. Chem.*, **A151**, 13 (1930), and other papers in this series.

determined experimentally and calculated. Included in the table are the corresponding parachor values for later reference.

TABLE XII

PARACHOR AND NULLPUNKTSVOLUME VALUES OF SOME TYPICAL ORGANIC COMPOUNDS

Compound	Nullpunktsvolume			P (using M. and P.'s Factors)		
	Calc.	Obs.	Δ	Calc.	Obs.	Δ
Methyl alcohol....	30.2	30.5	+0.3	85.4	88.3	+2.9
Ethyl alcohol.....	45.2	44.7	−0.5	125 4	126.8	+1.4
Ethane..........	41.6	40.0	−1.6	110.8	110.5	−0.3
Formic acid.......	29.5	29.1	−0.4	.....	.....	.....
Acetic acid.......	44.5	44.7	+0.2	130.6	131.2	+0.6
Acetone..........	55.9	55.2	−0.7	159.0	161.5	+2.5
Acetaldehyde.....	40.9	38.3	−2.6	.....	.....	.....
Ethyl ether.......	75.2	75.8	+0.6	211.3	211.7	+0 4
Stearic acid.......	.....	.....	.....	770 6	778.0	+7.4
Cyclohexanol.....	.....	.....	.....	255.4	254.9	−0.5
Benzene..........	67.2	69.3	+2.1	205.4	206.3	+0.6
Naphthalene......	100.4	102.5	+2.1	.....	.....	.....
Anthracene.......	133.6	135.8	+2.2	.....	....	....
Toluene..........	82.2	83.6	+1.4	245.4	246.2	+0.8
Xylene...........	97.2	98.2	+1.0	285.4	283.8	−1.6
Mesitylene.......	112.2	111.2	−1.0	321.8	320.8	−1.0
Ethylbenzene.....	97.2	97.7	+0.5	.....	.....	.....
Propylbenzene....	112.2	116.2	+4.0	.....	....	.....
Benzaldehyde.....	81.5	82.4	+0.9	253.6	255.1	+1 5
Methyl salicylate..	.....	.....	.....	321.8	323.7	+1.9

It will be noticed that the calculated nullpunktsvolumes for aliphatic compounds are for the most part greater than the observed values; for aromatic compounds, the calculated values are uniformly lower than the observed. Additional constitutive factors which take account of ring formation, steric effects, etc., must be introduced in the calculation if better agreement between observed and computed values is to be realized.

The influence of the crystal form of solid substances on their nullpunktsvolumes is illustrated in Fig. 5, which graphically presents the nullpunktsvolumes of members of two homologous series. The alternating effect of the even and odd members of the series, which is characteristic of melting points, is also found in the volume values of solid com-

pounds. The molecular volumes and parachors of corresponding liquid substances do not show this alternating effect.

The *molecular volume* of liquids has been studied by Kopp. After searching for the most suitable conditions, he chose to determine the densities near the boiling points. According to Guldberg's rule, the

Dicarboxylic acids   FIG. 5   n-Paraffin hydrocarbons
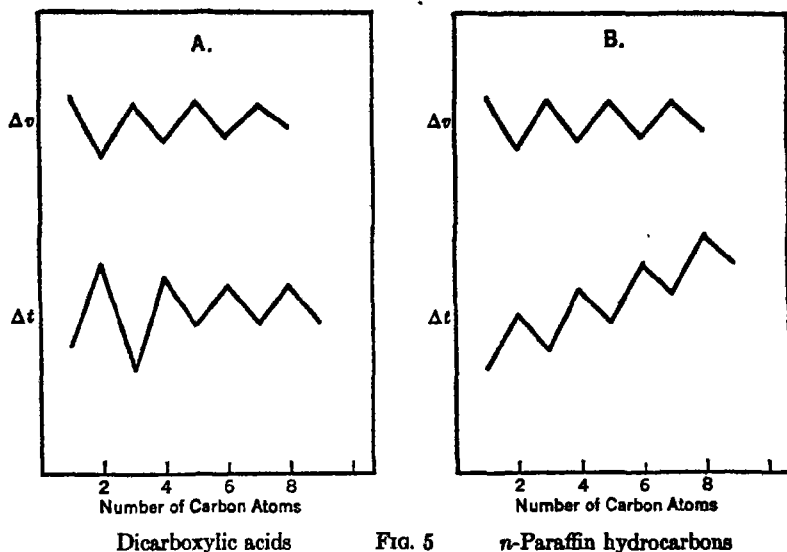
(Δv — difference in nullpunktsvolume; Δt — difference in melting point.)

Taken from Bilts, Fischer and Wünnenberg, *Z. physik. Chem.*, **A151**, 13 (1930). (Courtesy of publishers.)

boiling points are numerically about two-thirds of the critical temperatures and therefore represent corresponding states. The following table gives the atomic constants as determined by Kopp.

TABLE XIII[*]

C		11.0
H		5.5
—O—	(linked to two C atoms)	7.8
=O	(linked to one C atom)	12.2
—S—	(linked to two C atoms)	22.6
=S	(linked to one C atom)	28.6
Cl.		22.8
Br		27.8
I		37.5

[*] Smiles, "The Relation between Chemical Constitution and Physical Properties," Longmans, Green & Co., London (1910).

Constitutive factors were evaluated for unsaturation, chain length, isomerism, etc.

The determination of d at corresponding states eliminates variations caused by intermolecular forces. The elimination of intermolecular forces is also accomplished by measuring the partial molal volumes of substances in dilute solutions of inert solvents. From measurements of partial molal volumes of various compounds in the same solvent and at the same dilution, Traube [43] concluded that in dilute solutions molecular volumes or molecular solution volumes, as he termed them, could be calculated from atomic constants. Later work [44] has applied this principle of molecular volumes observed in dilute solutions to the calculation of additive constants with a considerable degree of success.

An excellent example of the utility of the systematic study of a physical property for purposes of predicting and checking numerical values of the property is afforded by a study of the molecular volumes of normal alkanes as reported by Calingaert and Hladky [7] and others.[45]

Another method of taking account of intermolecular forces in calculating additive and constitutive factors of molecular volumes has been developed by Sugden.[46] Macleod observed the empirical relationship $\gamma = C(D - d)^4$, where γ is the surface tension of a compound, D its density in the liquid form, d its density in the vapor form, and C a constant. Sugden multiplied this expression by M, the molecular weight, and obtained

$$\frac{M\gamma^{\frac{1}{4}}}{D - d} = MC^{\frac{1}{4}} = P$$

where P is called the *parachor*.

At the critical temperature, the surface tension of a compound is zero, since the intermolecular attractive forces are equal to the thermal kinetic forces. Since $\gamma = 0$ for all compounds at their critical temperatures, molecular volumes measured at these temperatures are more strictly comparable when additive and constitutive factors are being investigated. Sugden has pointed out that the parachor for most substances is equal to 0.77 times the molecular volume at the critical temperature, i.e., $P/V_c = 0.77$.[*]

In Table XIV are found data given by Sugden. In this table, P is the experimentally observed parachor, V_c is the molecular volume at the

[43] Traube, *Samml. chem. chem.-tech. Vorträge*, **4**, 255 (1899).

[44] Cohn, McMeekin, Edsall, and Blanchard, *J. Am. Chem. Soc.*, **56**, 784 (1934).

[45] Calingaert, Beatty, Kuder, and Thomson, *Ind. Eng. Chem.*, **33**, 103 (1941).

[46] Sugden, "The Parachor and Valency," Routledge and Sons, Ltd., London (1930).

[*] It would seem at first sight that P should equal zero at the critical temperature, since $\gamma = 0$. This is not necessarily true, because $D - d$ also equals zero, and the quotient of $\gamma^{\frac{1}{4}}$ and $D - d$ is indeterminate. There is therefore no reason to expect a sudden discontinuity in the value of P as the temperature approaches the critical temperature.

critical temperature, and a is the molecular area as calculated from collision numbers.

TABLE XIV

PARACHORS, CRITICAL VOLUMES, AND MEAN COLLISION AREAS

Compound	P	V_c	P/V_c	$a(\times 10^{16})$ cm^2	$P^{\frac{3}{4}}/a$
Propyl acetate	256.1	345.3	0.74	17.11	2.30×10^{16}
Ethyl acetate	217.1	286.3	0.76	15.01	2.41
Methyl acetate	177.0	227.8	0.78	12.80	....
Methane	73.2	99	0.74	7.72	2.27
Ethane	110.5	143	0.77	.....	....
Ethylene	99.5	127	0.78	10.81	2.16
Methyl chloride	110.4	136	0.81	9.67	2.38
Ethyl chloride	149.4	194	0 77	11.94	2.36
Methyl formate	138.6	172.0	0.81	17.11	2.30
Ethyl propionate	254.8	344.3	0.74	.....	....
Diethyl ether	211.7	281.9	0.75	.....	....
Benzene	206.3	256.1	0.81	13.83	2.52
Chlorobenzene	244.5	307.8	0.80	.....	....
		Mean	0.77	Mean	2.32

The analysis of parachor values has proved highly successful. Much work has been done on the numerical values for the various structural units. Sippel [47] has prepared a set of atomic constants for non-cyclic compounds and a series of increment values for cyclic compounds; reasonably satisfactory values are obtained by his method, although no reference is made to the linking of the various atoms. Mumford and Phillips [48] have revised Sugden's constants to correct errors in the parachors of higher homologs and have considerably improved the accuracy of the calculated values, as compared with those observed (Table XII). Vogel [6] has also recently revised some of Sugden's original values.

Parachor values for some structural units, as calculated by various investigators, are collected in Table XV. A computation of the parachor value for benzene illustrates the use of the data.

Benzene has 6 carbon atoms, 6 hydrogen atoms, and 3 double bonds; it is a six-membered ring. If Mumford and Phillips' values are used, as given in the table, the calculation is:

$$P = (6 \times 9.2) + (6 \times 15.4) + (3 \times 19) + 0.8 = 205.4$$

The value computed from the experimental observations is 206.3.

[47] Sippel, Ber., 63, 2185 (1930).
[48] Mumford and Phillips, J. Chem. Soc., 2112 (1929).

<div align="center">

TABLE XV

ATOMIC AND STRUCTURAL CONSTANTS FOR CALCULATION OF THE PARACHOR
</div>

	S.*	M.P.	V.
CH_2	39.0	40.0	40.3
C	4.8	9.2	11.5
H	17.1	15.4†	14.4
O	20.0	20.0	....
O_2 (Ester)	60.0	....	....
N	12.5	17.5	....
S	48.2	50.0	....
F	25.7	25.5	....
Cl	54.3	55.0	....
Br	68.0	69.0	....
I	91.0	90.0	....
Singlet bond	....	−9.5	....
Double bond	23.2	19.0	....
Triple bond	46.6	38.0	....
3-Membered ring	16.7	12.5	....
4- " "	11.6	6.0	....
5- " "	8.5	3.0	....
6- " "	6.1	0 8	. ..
7- " "	....	−4 0	. ..

* S. = Sugden; M.P. = Mumford and Phillips; V. = Vogel.

† H linked to C; for H linked to O or N the value is 10.0 or 12 5, respectively.

Langmuir [49] and Harkins [50, 51] developed methods for *spreading organic compounds on surfaces* where they form coherent monomolecular layers. From the known amounts of the substance, G, the extent of the surface, F, and the molecular weight, the space occupied by a single molecule, f, can be calculated by means of the equation

$$f = \frac{FM}{NG}$$

where N = Avogadro's number. Langmuir found, for a series of fatty acids and alcohols spread on water, that the surface occupied was independent of the number of carbon atoms in the chain and equaled about 23×10^{-16} cm.^{2} per molecule, while the thickness of the layer was proportional to the chain length and increased 1.2 to 1.3 Å per CH_2 group. It was concluded from these data that all molecules were arranged on the surface of the water so that the hydrocarbon chains were erect and had a zigzag form.

Additional methods for the determination of the sizes of molecules, which are, however, of less importance for organic chemistry, are those

[49] Langmuir, J. Am. Chem. Soc., 39, 1848 (1917).

[50] Harkins, Brown, and Davies, ibid., 39, 354 (1917).

[51] Harkins, Davies, and Clark, ibid., 39, 541 (1917).

based on the determination of b in the van der Waals equation, on measurements of the internal friction of gases and liquids, and on measurements of the collision areas of gases.

VISCOSITY

The viscosity, η, of a liquid is a measure of the resistance set up by intermolecular attractive forces to the passage of one molecule past another. Viscosity data are therefore subject to the same difficulties of analysis as those of other properties which depend upon the mutual attraction of molecules. Viscosity is strongly dependent upon temperature, and measurements designed to separate the additive and constitutive components of this property must be carried out under conditions which are equivalent for all compounds.

Some measure of success has been obtained in efforts to prepare series of numerical constants from which molecular viscosities can be calculated. Dunstan and Thole [62] have developed the formula $\log \eta = aM + b$, where M is the molecular weight, a a general constant, and b a constant characteristic of an homologous series. By means of this formula, logarithmic increments for various atomic groups have been calculated.

TABLE XVI

INCREMENT FACTORS FOR VISCOSITY PREDICTION (DUNSTAN AND THOLE)

CH$_2$	0.107	Alcoholic O	2.12
Aliphatic H	0.934	Double bond	1.847
Aliphatic O	0.098	n-Carbon	−1.761
Iso-Carbon	−0.030		

Fluidity, which is the reciprocal of the viscosity, has been shown [63] to be a function of the vapor pressure. At high vapor pressures the relationship is nearly linear, and from data taken in the range where this linearity holds it has been possible to calculate increment factors for fluidity.

TABLE XVII

INCREMENT FACTORS FOR PREDICTION OF FLUIDITY (BINGHAM)

	Fluidity		Fluidity
Carbon	−95	Hydrogen	59
Oxygen	24	Iso-linkage	−76
Double bond	114	Sulfur	76
Chlorine	109		

[62] Dunstan and Thole, *J. Inst. Petroleum Tech.*, **4**, 191 (1918).
[63] Bingham and Spooner, *J. Rheol.*, **3**, 221 (1932).

Prasad [54] has observed regularities in the temperature-viscosity relationships of members of homologous series. He finds indications that the odd and even members of the series fall into separate groups.

Staudinger, in his studies on the viscosities of solutions of high-molecular-weight compounds,[55] has introduced the term *specific viscosity*, η_{sp}, which is defined as the increase in viscosity which is produced in a solvent by dissolving a unit amount of a substance in a unit volume of the solvent. He has made use of the expression $\eta_{sp}/C = KL$, where C is the concentration of a primary molal solution (1.4 per cent = $CH_2/1000$), K a constant, and L the length of the carbon chain in Ångström units of the material being investigated. For simple, normal organic compounds, such as paraffins and fatty acids, the specific viscosity of a primary molal solution may be expressed as η_{sp} (1.4 per cent) $= ny + x$, where n is the number of carbon atoms in the chain, y the viscosity of a single carbon atom, and x that of oxygen. For many compounds, y is approximately constant. The plot of η_{sp}/C against the number of carbon atoms for several homologous series shows that the slopes of the lines are approximately the same; the intercepts on the η_{sp}/C axis therefore give the values of x for each series. Some of Staudinger's results are shown in Fig. 6.

Huggins,[56] from general considerations, derived a formula which accounts quantitatively for the viscosities of solutions of the paraffins. The equation is of the same form as Staudinger's. Random distribution about single bonds is assumed, and the effects of restricted rotation, as well as of the size of the solvent molecule, find a rational interpretation.

Staudinger has extended his concept of additive group viscosity values to considerations of the viscosities of high-molecular-weight polymers such as cellulose and its derivatives. From his data he has calculated the molecular weights of such compounds and has computed the numbers of structural units in the polymeric chains. Some of his results do not agree with values obtained by other methods of measurement.

Flory [57] measured the viscosities of molten linear polyesters having average molecular weights in the range 200 to over 10,000. He found that the logarithm of the viscosity is precisely a linear function of the square root of the weight average chain length throughout this range.

[54] Prasad, *J. Indian Chem. Soc.*, **10**, 143 (1933).

[55] Staudinger, "Die hochmolekularen organischen Verbindungen," Springer, Berlin (1932).

[56] Huggins, *J. Phys. Chem.*, **42**, 911 (1938); **43**, 439 (1939); *J. Applied Phys. U.S.S.R.*, **10**, 700 (1939).

[57] Flory, *J. Am. Chem. Soc.*, **62**, 1070 (1940).

Harms [58] has treated the relation between the viscosity of a liquid and the chemical constitution of its molecules. He showed the influence of the intermolecular forces and the temperature agitation on the molecules and explained the change of the viscosity with varying temperatures and pressures.
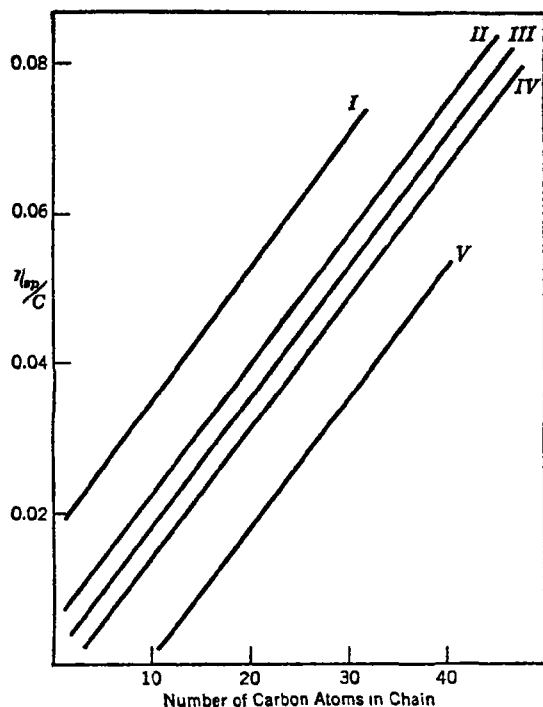
FIG. 6.—Viscosity changes in homologous series.

I = *n*-fatty acids in pyridine; *II* = *n*-fatty acids; *III* = *n*-fatty acid esters (ethyl); *IV* = *n*-paraffins; *V* = *n*-alcohols.

Eyring,[58a] Ewell and Eyring,[58b] and Kincaid, Eyring, and Stearn [58c] have treated viscosity from the standpoint of modern reaction kinetics; the molecules, shearing past each other, undergo a series of reactions involving the intramolecular forces. The results arrived at show good agreement with the experiment and justify this fundamental treatment of the subject.

[58] Harms, *Z. physik. Chem.*, **B44**, 14 (1939)
[58] (a) Eyring, *J. Chem. Phys.*, **4**, 283 (1936); (b) Ewell and Eyring, *ibid.*, **5**, 726 (1937); (c) Kincaid, Eyring, and Stearn, *Chem. Rev.*, **28**, 301 (1941).

REFRACTIVE INDEX

The *refractive index*, n, of a substance is the ratio of the velocity of light in vacuum to the velocity of light in that substance. Since the latter quantity is diminished by the interaction of the light waves with the electrostatic and electromagnetic fields in the molecules, n depends upon the polarizability of the atoms, which in turn is modified by their arrangement in the molecules. This relationship is well understood and formulated in the dispersion formula.[59] On account of thermal expansion effects, the refractive index varies with the temperature, while the specific refractivity, r, as proposed by Lorentz and Lorenz,[60]

$$r = \frac{n^2 - 1}{n^2 + 2} \cdot \frac{1}{d}$$

The density, d, is very nearly independent of pressure and temperature. The product of r with the molecular weight is called the *molecular refraction*. According to Maxwell's theory, the square of the refractive index, for infinitely long waves, is identical with the dielectric constant, ϵ. In so far as the condition $n^2 = \epsilon$ is fulfilled for the wavelength at which n is determined, the molecular refraction becomes identical with the total molecular polarization (p. 1753).

$$P = \frac{\epsilon - 1}{\epsilon + 2} \cdot \frac{M}{d}$$

Another useful function has been suggested by Dale and Gladstone:

$$r = \frac{n - 1}{d}$$

Though this formula also gives constant and analyzable values, it is theoretically less significant than the above-mentioned Lorenz-Lorentz equation.

The refractive index varies with the wavelength of light, and it is therefore necessary to designate the wavelength at which the measurement is made. This is customarily done by using a subscript; thus, n_α indicates the refractive index as observed with the hydrogen α line; M_D the molecular refraction as measured with the sodium D line, and $M_{546.1}$ the molecular refraction at λ 546.1 mμ (the mercury green line). The difference between molecular refractions measured at two wavelengths is called the *molecular dispersion*.

[59] Page, "Introduction to Theoretical Physics," Van Nostrand, New York (1928), p 439.

[60] (a) Lorentz, *Wied. Ann.*, 9, 641 (1880); (b) Lorenz, *ibid.*, 11, 70 (1880).

The mathematical analysis of data from measurements of molecular refractions has led to the assignment of values for the atoms which constitute the compounds, termed *atomic refractions*. Where the atoms are bound by other than single linkages, e.g., C=C, additional values, increments, are assigned for these linkages. In Table XVIII are given some

TABLE XVIII

ATOMIC FACTORS FOR CALCULATING MOLECULAR REFRACTIONS

Atom or Group	H_α	D	H_β	H_γ	$H_\gamma - H_\alpha$
—CH₂—	4.598	4.618	4.668	4.710	0.113
C	2.413	2.418	2 438	2.466	0.056
H	1.092	1.100	1.115	1.122	0.029
O(carbonyl)	2.189	2.211	2.247	2.267	0.078
O (ether)	1.639	1.643	1.649	1.662	0.019
O (hydroxyl)	1.522	1.525	1 531	1.541	0.015
Cl	5 933	5.967	6.043	6.101	0.168
Br	8.803	8.865	8 999	9 152	0 340
I	13.757	13.900	14.224	14.521	0.775
N (*pri.*-amine)	2.309	2.322	2.368	2.397	0.086
N (*sec.*-amine)	2.475	2.499	2 561	2.603	0.119
N (*tert.*-amine)	2.807	2 840	2.940	3.000	0.186
S (mercaptan)	7.63	7.69	7.83	7.98	0.35
CN	5 434	5.459	. ..	.....	.....
C=C (double bond)	1.686	1.733	1.824	1.893	0.200
C≡C (triple bond)	2.328	2.398	2.506	2.538	0.171

of these factors determined by Auwers and Eisenlohr.[61] The headings in the columns indicate the wavelengths of light used in the measurements. The values for the molecular dispersions are given in the last column. The addition of these values leads in many cases to molecular refractions in agreement with the observed values. However, in other cases, differences are found between the calculated and the observed values. These differences have in turn been analyzed and allocated to certain constitutive factors. These so-called *exaltations* of the molecular refractions are indicated as EM, and the exaltations for the molecular or specific dispersions by $E(M_\gamma - M_\alpha)$ or $EM_{\gamma-\alpha}$.
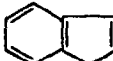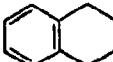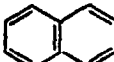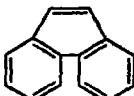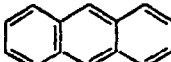
While the increment (C=C) (Table XVIII) adequately represents any number of isolated double bonds in the molecule, the presence of conjugation between two or more double bonds gives rise to an exaltation. Examples of these effects were presented in Table XV, p. 1741, of the first edition of this work. The exaltation increases as the length of the conjugated chain becomes greater, provided that the chain is

[61] Auwers and Eisenlohr, *Ber.*, **43**, 806 (1910), and subsequent articles in this journal, *Ann.*, and *J. prakt. Chem.*

unsubstituted. For a given chain, the exaltation decreases progressively as the carbon atoms are progressively substituted with R groups. Exaltation is greatest for a linear conjugated system and decreases as the system becomes more and more branched.

In a study of the exaltation of methylbutadiene derivatives,[62] it was observed that the shifting of the methyl substituents to the center of the conjugated system resulted in a marked lowering of the exaltation. The exaltation is the result of an increased polarizability of the system. Where this is offset by resonance, as in benzene, furan, thiophene, pyrrole, and cyclopentadiene, the exaltations become negligible. Although these aromatic compounds show practically no exaltation, the polycyclic compounds derived by their combination have decided exaltations, as shown by Table XIX. This fact has been interpreted as an indication that only one ring in such compounds is truly aromatic, the other rings producing the exaltation characteristic of normal unsaturated conjugation.

TABLE XIX

RELATION BETWEEN CYCLIC STRUCTURE AND REFRACTION

	Benzene	Cyclopentadiene		Indene
$EM_{\gamma-\alpha} =$	+6 per cent	−0.5 per cent		+35 per cent

	Benzene	Cyclohexene		Tetrahydronaphthalene
$EM_{\gamma-\alpha} =$	+6 per cent	0.0 per cent		+16 per cent

	Benzene	Naphthalene	Phenanthrene	Anthracene
$EM_{\gamma-\alpha} =$	+6 per cent	+60 per cent	+90 per cent	+170 per cent

DIPOLE MOMENT

The *electric moment* or *dipole moment*[63] between the centers of gravity of the positive and negative charges is the product of charge, e, and distance, l. Since it is zero for all molecules in which the centers of gravity coincide, the presence or absence of a moment can give definite clues to the constitution of compounds. Thus, the finite moment of the symmetrical ethers, like dimethyl ether, proves the angular nature

[62] Farmer and Warren, *J. Chem. Soc.*, 3221 (1931).
[63] Debye, "Polar Molecules," Chemical Catalog Co., New York (1929).

of the oxygen valences; that of the trialkylamines, like trimethylamine, argues against their planar structure. The absence of a moment for methane confirms the tetrahedral structure of this compound; the moment zero of the dichloroethylene of b.p. 48° shows that this isomer is the *trans* compound, etc.

The determination of the dipole moment is based upon the measurement of the dielectric constant, ϵ. The dielectric constant of a substance without a dipole moment is determined only by the internal polarization, i.e., a shift of the electrons, electron polarization, and of the atoms, atom polarization. The atom polarization, which usually can be neglected,[64] will not be considered in the following. A molecule with a dipole moment, on the other hand, tends to become oriented in the direction of an electric field, and the degree of orientation is dependent upon the size of the dipole. This orientation polarization is temperature-dependent, because thermal agitation disturbs the arrangement of the molecules, while electron and atom polarization are temperature-independent. Hence, the orientation polarization can be separated as the temperature-dependent term of the dielectric polarization P:

$$P = \frac{\epsilon - 1}{\epsilon + 2} \cdot \frac{M}{d} = A + \frac{B}{T}$$

The orientation polarization is connected with the turning of molecules of considerable inertia and has an appreciable time of relaxation, at variance with the negligible time of relaxation of the electron polarization. Hence, only the latter responds to alternating fields of high frequency, i.e., light waves, while waves of radio frequency excite both electron and orientation polarization. The orientation polarization can therefore also be determined as the difference between the molecular polarization obtained from dielectric constants measured with long waves and the molecular refractions calculated with the Lorenz-Lorentz equation.

From the orientation polarization of one mole of substance, the orientation polarization of the individual molecule and the dipole moment are calculated. The moments of the common organic molecules have values between 0 and 10×10^{-18} e.s.u. This is in agreement with expectation, since the charges involved are of the order of magnitude of 10^{-10} e.s.u., and the distances in molecules of the order of 10^{-8} cm. The debye (D) is defined as the unit of dipole moment, and is equivalent to 10^{-18} e.s.u.

Dipole moments are vector quantities. The direction of a moment in space is determined by the line which connects the centers of gravity

[64] However, see LeFèvre and Vine, *J. Chem. Soc.*, 1878 (1938).

of the positive and negative charges, and the total dipole moment of a molecule may be made up of partial moments. For example, CH_4 has the moment zero, because the centers of gravity of the nuclei and of the electrons coincide, or, from another viewpoint, because the four component C—H moments are equal and opposite in direction. CH_3Cl has a finite electric moment (1.86), which is the resultant of the C—H and C—Cl moments, and the introduction of additional chlorine atoms causes a decrease in the moment (CH_2Cl_2, 1.59; $CHCl_3 \sim 1.0$) until zero is reached with the symmetrical CCl_4.

In a series of homologous compounds which have a single polar group, e.g., the n-alcohols and n-acids, the moments of the members of the series are approximately constant. Thus, the moments found for the first ten normal alcohols all lie between 1.6 and 1.7, with the average moment 1.67. In the fatty acid series, the first member, formic acid, has a greater moment than the higher members, but the latter have approximately constant values. The moments of acetic, propionic, and stearic acids have been found to be identical within the experimental error of measurement.[65] In Table XX are given the moments of some typical organic series which contain single polar groups; also included are moment values for a few benzene derivatives. The phenols and aromatic ethers show the same moments as the corresponding aliphatic compounds.

<div align="center">

TABLE XX

MOMENTS OF ORGANIC COMPOUNDS

</div>

Compound	Moment	Compound	Moment
n-Hydrocarbons	0.0×10^{-18}	Mercaptans	1.3×10^{-18}
n-Alcohols	1.67	Sulfides	1.5
Ethers	1.2	Cyanides	3.4
Alkyl chlorides	2.0	Nitroparaffins	3.1
Esters	1.8	Nitrites	2.2
Primary amines	1.3	Nitrates	2.9
Secondary amines	1.0	Benzene	0.0
Tertiary amines	0.76	Phenol	1.70
Ketones	2.7	Anisole	1.20
Formic acid	1.2	Bromobenzene	1.50
n-Acids	0.8		

Group and bond moments have been assigned to various structural units. The values in Table XXI are those given by Sutton;[66] they vary with the nature of the alkyl radicals to which the atoms or groups are attached.

[65] Wilson and Wenzke, J. Chem. Phys., 2, 546 (1934).
[66] Sutton, Proc. Roy. Soc. (London), A133, 668 (1931).

TABLE XXI

COMPONENT FACTORS FOR CALCULATION OF MOMENTS (SUTTON)

Group	$m_{aromatic}$	$m_{aliphatic}$	$m_{ethylenic}$	m_e
—CH₃	+0.45	0.0	....	+0.45
═O	−1.06	−1.29	....	+0.23
—NH₂	+1.55	+1.23	....	+0.32
—Cl	−1.56	−2.15	−1.66	+0.59
—Br	−1.52	−2.21	−1.48	+0.69
—CH₂Cl	−1.82	−2.03	−2.00	+0.21
—I	−1.27	−2.13	....	+0.88
—OH	−1.7	−1.83	....	+0.15
—OCH₃	−1.2	−1 16	....	0
—CHO	−2.75	−2 46	....	−0.29
—COCH₃	−2.97	−2.79	....	−0.18
—CO₂CH₃	−1.93	−1.71	....	−0.22
═C═O	−3 04	−2.76	....	−0.28
—C≡N	−3.89	−3.46	....	−0.43
—NO₂	−3.93	−3.05	....	−0.88

Ketelaar [67] has given a quantum-mechanical evaluation of this effect. Further variations, depending on the radical to which the polar group is attached, are shown in Table XXII. The moments increase, in general, as the carbon atom attached to the polar group becomes progressively substituted, while substitution in the chain on a carbon not directly attached to the polar group has little effect on the moment.

TABLE XXII

MOMENTS OF ALIPHATIC BROMIDES AND ALCOHOLS

Compound	Moment	Compound	Moment
n-Butyl bromide	1.88×10^{-18}	n-Amyl alcohol	1.63×10^{-18}
sec.-Butyl bromide	2.12	sec.-Amyl alcohol-(2)	1.66
tert.-Butyl bromide	2.21	sec.-Amyl alcohol-(3)	1.64
Isobutyl bromide	1.97	tert.-Amyl alcohol	1.83
		Isoamyl alcohol	1.81

Sutton divides the dipole moment of a polar compound into three components: a primary moment which is characteristic for the substituent; the moment induced electrostatically on the rest of the molecule; and the moment due to the electromeric shift of electrons. The electromeric effect, m_e in Table XXI, indicates the orienting nature of the group; i.e., a positive m_e—electromeric shift of electrons into the radical —means an *ortho-para* directing group, while a negative m_e signifies a

[67] Ketelaar, *Rec. trav. chim.*, 58, 311 (1939).

meta directing group. The magnitude of m_s is a relative measure of the orienting strength of the group.

A special instance of the inductive effect is the mutual interaction of strongly polar atoms and groups which is illustrated below by *o*-dichlorobenzene.

The addition of the vector dipoles μ_1 and μ_2 of groups in the *ortho*, *meta*, or *para* positions in benzene is graphically represented in Fig. 7. The moments μ_o, μ_m, and μ_p are the resultant molecular moments when
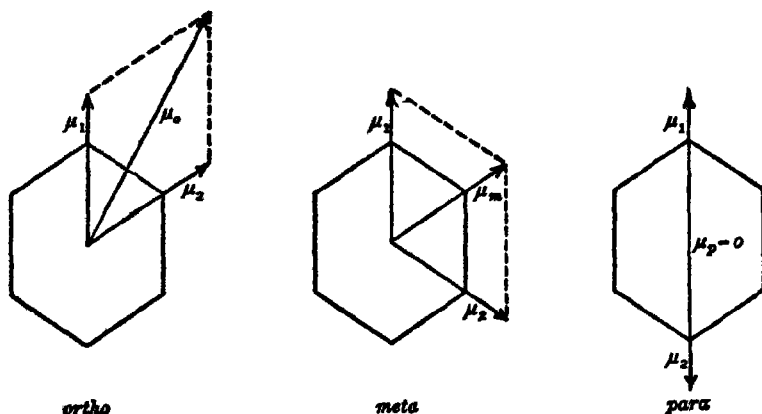
FIG. 7.—Representation of dipole forces in disubstituted benzene derivatives.

the groups of moment μ_1 and μ_2 are in the *ortho*, *meta*, and *para* positions, respectively.

Group moments are vectorially added by use of the equation
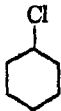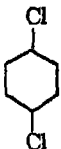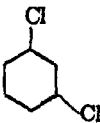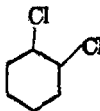
$$\mu = \sqrt{\mu_1^2 + \mu_2^2 + 2\mu_1\mu_2\cos\theta}$$

where μ_1 and μ_2 are the moments of the groups and θ is the angle of separation of the dipoles having these moments. Similar circumstances prevail in the aliphatic series, where in general the tetrahedral angle for carbon is confirmed by the measurements and the rotation of angular groups about single bonds must be taken into account.[68] In benzene derivatives, θ becomes 60° for the *ortho*, 120° for the *meta*, and 180° for the *para* configuration. If μ_1 and μ_2 are identical, the resultant moments of *ortho*, *meta*, and *para* configurations become $\mu\sqrt{3}$, μ, and 0, respectively.

The observed moment of the *ortho* compound is considerably smaller than that calculated, largely because of a mutual polarization of the

68 Williams, Z. physik. Chem., A138, 75 (1928).
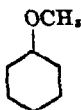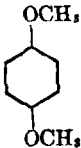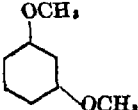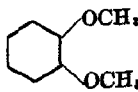
TABLE XXIII

μ Observed	1.55	0.00	1.48	2.25
μ Calculated	—	0.00	1.55	2.67
				2.33 (with Smallwood and Herzfeld correction)

component moments; this becomes evident in the better agreement with experiment of the calculated value, which, according to Smallwood and Herzfeld,[69] takes this induction into consideration. A spreading of the angle between C—Cl bonds because of steric repulsion of the chlorines might be responsible for the residual difference. With methylene chloride, which also has a dipole moment smaller than that calculated by the addition of the two C—Cl vectors, the spreading of the Cl—C—Cl angle makes a larger contribution to the difference between the observed values and those calculated with the component factors for aliphatic chlorine and the tetrahedral angle. The spreading is confirmed in this case by measurements of electron diffraction.

If the substituents are not axially symmetrical, the order of the moments may be reversed:[70]

TABLE XXIV

μ =	1.23	1.8	1.6	1.3

This is due to the fact that in the para compound the angular —OCH₃ groups do not interfere with each other—free rotation, random distribution—whereas in the ortho compound they interact in a way which diminishes the moment.

Many general observations have been made. Wolf and Gross[71] have shown that the same type of alternation exists in the moments of adjacent members of homologous series as is shown in melting points,

[69] Smallwood and Herzfeld, J. Am. Chem. Soc., 52, 1919 (1930).
[70] Weissberger and Sängewald, Physik. Z., 30, 792 (1929).
[71] Wolf and Gross, Z. physik. Chem., B14, 305 (1931).

specific heat, etc. Smyth and Walls [72] have interpreted some of their data to mean that long-chain molecules are fairly rigid in structure. These investigators have demonstrated that *normal* and *iso* aliphatic compounds containing a polar group show the same moments if the branching occurs at least two atoms from the polar group. It has been found [73] that, if a carbon chain contains unsaturated conjugated linkages, the dipole effect of a polar group attached to the chain is transmitted along the chain. The connection between dipole moment and intermolecular forces and the properties determined by them has been studied by van Arkel.[5]

The use of dipole-moment data in attacking problems of structural organic chemistry has been fruitful in a number of cases. From the molecular moments and the knowledge of individual group moments, taking into account the interaction between the substituent and the other groups, it has been possible to calculate with great accuracy the valence angles of some atoms like oxygen and sulfur.[74] As mentioned before, it is due to these angles that certain *para*-disubstituted benzene derivatives, such as the diethyl ether of hydroquinone and the dimethyl ester of terephthalic acid, have finite dipole moments.[75] The structures of a number of *cis-trans* isomers have been definitely established because of the fact that *cis* forms have appreciable moments, while the corresponding *trans* forms show much smaller or zero moments. The fact that tetranitromethane has zero moment proves that all four nitro groups are identical and are located at the corners of a tetrahedron. Dipole-moment measurements of a number of tetra-substituted methanes show that in none is it necessary to assume a pyramidal structure for such molecules, as has sometimes been postulated. An investigation of the mono- and dichloronaphthalenes proved that the naphthalene system is planar, that the C—Cl bonds are directed from the center of the ring, and that moments can be calculated in good agreement with the observed ones if the polarization of the system, a small electromeric effect, and the Smallwood-Herzfeld effect are considered.[76]

The problem of restriction of rotation about the C—C single bond has been treated. If free rotation exists, the moments of d-, l-, and meso-forms should be identical; if free rotation does not exist, the moment of the d- and l-form might be different from that of the meso-form. The dipole moment of diethyl d-tartrate is 3.12×10^{-18} e.s.u., while the

[72] Smyth and Walls, *J. Am. Chem. Soc.*, **53**, 527 (1931); **54**, 2261 (1932).

[73] Farmer and Warren, *J. Chem. Soc.*, 1302 (1933).

[74] (a) Glasstone, "Recent Advances in Physical Chemistry," 2nd ed., Blakiston's Son and Co., Philadelphia (1933), p. 135; (b) Coop and Sutton, *J. Chem. Soc.*, 1869 (1938).

[75] Debye, "Polar Molecules," Chemical Catalog Co., New York (1929).

[76] Hampson and Weissberger, *J. Chem. Soc.*, 393 (1936).

moment of diethyl *meso*-tartrate is higher, 3.66×10^{-18} e.s.u.[77] It appears strange that the electric moment of the *meso*-form, which, by its lack of optical activity, is revealed to be the more symmetrical form, is higher than that of the active form. However, in the tartrates, this is caused by the presence of angular groups, for reasons similar to those given in connection with the moments of the dimethoxybenzenes.

If only axially symmetrical groups are present, as in the stilbene dichlorides, the more symmetrical *meso*-form has the lower (1.3) and the *dl*-form the higher (2.71) moment.[78]

The moments of the *para*-substituted diphenyls confirm the stretched formula of this compound, because they are identical with the moments of the corresponding benzene derivatives. The size of the moment of *o,o'*-dichlorodiphenyl indicates that in this compound the random distribution (free rotation) about the diphenyl link is interfered with, not only by the inner molecular electrostatic forces and by the steric interference of the chlorines, but also to a considerable extent by the mutual attraction of the chlorine atoms through London forces.[78]

The controversy as to the structures of the isomeric oximes between the original Hantzsch-Werner theory and that of Meisenheimer was finally settled in favor of the latter, by the measurement of the moments of the oxime ethers.[79]

More recently, the investigation of resonance by dipole measurements has been successfully pursued.[79] *p*-Nitroaniline has an abnormally high dipole moment, 6.2 D, exceeding by about 0.7 D the sum of those of aniline, 1.52, and nitrobenzene, 3.95. This has been interpreted as being due to resonance between the forms,
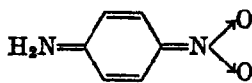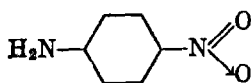
and necessitates that both the $-N\!\!<^O_O$ and the $-N\!\!<^H_H$ groups in the

second formula lie in the plane of the ring. If it were possible to deflect one or both of these groups out of that plane, the resonance, and therefore the dipole moment, should be diminished. There is evidence that in durene (symmetrical tetramethylbenzene) the methyl groups should have this effect on an NH_2 or NO_2 group placed between them, for in durene itself it has been shown, by x-ray analysis of the crystal, that the

[77] Wolf, *Trans. Faraday Soc.*, **26**, 315 (1930).

[78] Weissberger and Sängewald, *Z. physik. Chem.*, **B9**, 133 (1930); Weissberger, *J. Org. Chem.*, **2**, 245 (1937).

[79] Sidgwick, *Chem. Rev.*, **19**, 183 (1936).

repulsion of the methyl groups is sufficient to deflect them some $3°$ from their normal positions. It might be expected, therefore, that the moment of *p*-aminonitrodurene is less than that of *p*-nitroaniline, that this decrease becomes still greater by alkylation of the amino group in the durene derivative, and that a similar difference of moment exists between *p*-nitrophenol and *p*-nitrodurenol, and with the amino and the nitro compounds generally.[79] This question has been investigated by Hampson, Birtles, and Ingham,[80] who found that all these predictions of the resonance theory are confirmed by the observed dipole moments.

· Compounds or substances may or may not have permanent *magnetic moments*, just as compounds may or may not have permanent electric moments. The magnetic moment is calculated from measurements of the molecular magnetic susceptibility as dipole moments are calculated from molecular orientation polarization. Substances without a permanent magnetic moment are pushed out of a magnetic field, diamagnetism; substances with a permanent magnetic moment are drawn into the magnetic field, paramagnetism. The intensity of the force with which this takes place, usually measured by means of a balance, is called the intensity of magnetization, I, and the molecular magnetic susceptibility is defined by

$$\chi_{mole} = \frac{IM}{dH}$$

where H is the strength of the magnetic field and d and M are the density and the molecular weight of the compound measured.

The magnetic susceptibility is positive for paramagnetic substances and negative for diamagnetic substances. The absence of permanent magnetic moments in most organic compounds is due to the fact that all their electrons are paired in systems with antiparallel spins, while a permanent magnetic moment, paramagnetism, is due to odd electrons and is therefore characteristic of free radicals. Paramagnetism is temperature-dependent, because the orientation of the molecules with permanent magnetic moments in a magnetic field and their attraction into the field are disturbed by the temperature agitation of the molecules. The interpretation of the observed values is greatly assisted by the fact that each odd electron in a molecule contributes a certain amount to the total moment, which was calculated by Bohr. Diamagnetism, i.e., negative magnetic susceptibility, is due to the induction of inner-molecular moments by the outer field. These induced moments are not affected by the temperature agitation of the molecules, and diamagnetism is therefore temperature-independent.

[80] Birtles and Hampson, *J. Chem. Soc.*, 10 (1937); Ingham and Hampson, *J. Chem. Soc.*, 981 (1939).

The molecular diamagnetic susceptibility, which has been extensively investigated by Pascal,[81] shows phenomena very similar to the molecular refraction. It is an additive property with constitutive increments. Whereas the magnetic susceptibilities of the atoms are negative, the increment for the C=C double bond is positive. The suggestion that this is indicative of a radical nature of double bonds has been disproved, because the paramagnetic increment of di- and polyenes is temperature-independent.[82] The paramagnetism of free radicals was discovered by Taylor and Lewis.[83] Its quantitative evaluation gives a criterion for the amount of free radical present.[84] The method has been used in a series of interesting investigations by Marvel and his collaborators,[85] by Müller,[86] and by other authors.

Of the many problems treated, it may only be mentioned that compounds of the type

are diamagnetic and, therefore, quinonoid, as represented by the formula. However, the *meta* derivative,

which cannot exist in a quinonoid

form, is paramagnetic and, hence, a true biradical. The radical nature of semiquinones has been proved by determinations of the paramagnetism of these compounds.[87] With the metal ketyls, an investigation of their magnetic susceptibility showed that some of them exist as free

radicals,

others as the dimeric pinacolates,

and

others again as mixtures of the two.[86]

[81] See review by Auwers, *Jahrb. Radioakt. Electronik*, 17, 184 (1921).

[82] Müller and Dammerau, *Ber.*, 70, 2561 (1937).

[83] Taylor and Lewis, *Proc. Natl. Acad. Sci. U. S.*, 11, 456 (1925).

[84] Müller and co-workers, *Ann.*, 520, 235 (1935); 521, 89 (1936); Roy and Marvel, *J. Am. Chem. Soc.*, 59, 2622 (1937).

[85] (a) Marvel, Ginsberg, and Mueller, *J. Am. Chem. Soc.*, 61, 77 (1939); (b) Marvel, Mueller, and Ginsberg, *ibid.*, 61, 2008 (1939); (c) Marvel, Rieger, and Mueller, *ibid.*, 61, 2769 (1939); (d) Marvel, Mueller, Himel, and Kaplan, *ibid.*, 61, 2771 (1939).

[86] Müller, *Z. Elektrochem.*, 45, 593 (1939).

[87] Michaelis, Boeker, and Reker, *J. Am. Chem. Soc.*, 60, 202 (1938).

THE STRUCTURES OF ORGANIC COMPOUNDS AS DETERMINED FROM X-RAY DIFFRACTION MEASUREMENTS

The study of the arrangement of atoms in crystals of inorganic compounds has been successfully attacked by the use of x-rays. By studying the diffraction of x-rays from crystalline surfaces, it has been possible to calculate the distances between the planes of atoms in the crystals, just as it is possible to calculate the distance between rulings of a grating from light-diffraction measurements. The success of the x-ray diffraction method for determining the structure of a crystal depends on the degree of symmetry of its internal structure; in general, the more symmetrically arranged structural units are the more easily analyzed.

The applicability of the x-ray diffraction method for determining the molecular configurations of organic compounds has been somewhat limited because most organic substances are quite complex from a structural viewpoint and consequently do not exhibit high degrees of symmetry. Nevertheless, a great deal of valuable information has been obtained about the structures of very fundamental types of compounds. It must be pointed out that the investigator of the molecular structures of organic compounds has had considerable help in his work from the classical pictures of such compounds as postulated for many years by organic chemists; he has had certain clues as to the direction he should take in the analysis of his data.

The results which have been obtained from the study of the structures of organic compounds by x-ray analysis have been summarized in a number of publications.[85]

The structures of a number of halogenated aliphatic hydrocarbons have been determined with some degree of certainty. Iodoform, 1,2,3,4,5,6-hexabromo- and 1,2,3,4,5,6-hexachlorocyclohexane, hexabromoethane, *cis*- and *trans*-dichloroethylene, and 1,1- and 1,2-dichloroethane have thus been investigated. It was found that in all of them the structures which fit the data best show carbon to have valence angles corresponding to tetrahedral angles. The cyclohexane derivatives have structures which place the carbon atoms on a puckered ring. The distance between chlorine atoms in *cis*-dichloroethylene is 3.6 Å as compared to 4.1 Å for *trans*-dichloroethylene.

In the diamond crystal each carbon atom is surrounded by four others at a distance of 1.54 Å, placed at the corners of a tetrahedron. In graphite the carbon atoms in one plane form a series of interlocking hexagons, the distance from carbon to carbon being 1.42 Å.

[85] Hendricks, *Chem. Rev.*, 7, 431 (1930); Bragg and Bragg, "The Crystalline State," Vol. 1, Bell (1934); Bernal and Crowfoot, *Ann. Rept. Chem. Soc. (London)*, p. 379 (1938); Robertson, *Chem. Rev.*, 16, 417 (1935).

It is interesting that the structure of hexamethylenetetramine has been accurately determined and is identical with one accepted by organic chemists.

A number of aliphatic compounds has been investigated, a few in detail. Of the n-hydrocarbons, nonacosane, $C_{29}H_{60}$, has been carefully analyzed. It was found that the carbon chain is continuous and does not fold back on itself. The carbon atoms lie in a plane and have the zigzag structure demanded by the tetrahedral carbon atom. In the zigzag structure the distance between alternate carbon atoms was found to be 2.54 Å, and the C—C distance is very near 1.54 Å, which was found for diamond. Other hydrocarbons show an increase of 2.54 Å for every two CH_2 groups added to the molecule.

The gross structures of the n-aliphatic acids,[89] the n-aliphatic alcohols,[90] and the n-aliphatic dicarboxylic acids have been investigated. In all these series, the carbon atoms lie in a plane and have a zigzag arrangement. In both the acid and alcohol series, it was found that the addition of each successive CH_2 group caused an increase in the unit structure twice as great as that observed for the n-hydrocarbons. This is shown in Fig. 8, which is taken from tabulated data.[91] The
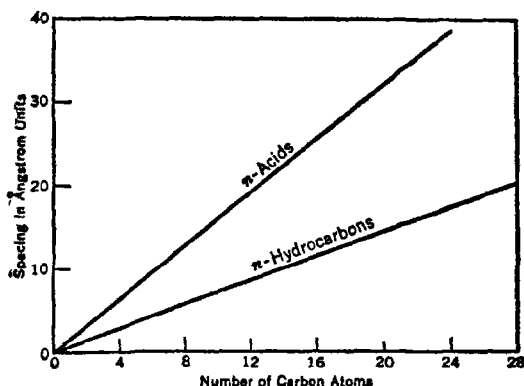
FIG. 8.—Regular increase in chain length of aliphatic homologs.

From Ewald and Harmann, loc. cit. (Courtesy of publishers.)

facts just cited argue that there are two molecules per unit structure for the alcohols and acids. In the fatty acid series, the unit spacings for the even carbon compounds are nearly equal to the spacings of the next higher odd carbon compounds. The addition of two CH_2 groups to an

[89] Francis, Piper, and Malkin, Proc. Roy. Soc. (London), A128, 214 (1930).
[90] Wilson and Ott, J. Chem. Phys., 2, 231 (1934).
[91] Ewald and Harmann, "Strukturbericht, 1913-28," Akad. Verlag., Leipzig (1931), p. 684.

even member of the dicarboxylic acid series causes an increase in unit spacing equal to that for the hydrocarbons; the addition of CH_2 groups to an odd member produces a change twice as great as that for the hydrocarbons.

Müller [92] has discussed the effect of the zigzag structure of the hydrocarbon chain upon the properties of the even and odd members of the dicarboxylic acid series. He has concluded that the alternating properties of this series can be explained by their structures; owing to the arrangement of the zigzag chain for the hydrocarbons, n-alcohols, or n-acids, the symmetry properties of the odd and even members of the series are different.

The structures of a number of aromatic compounds have been determined by an elegant method using a two- or three-dimensional Fourier series. This method of attack often leads to precise allocations of the atoms in crystals and to the electron density distributions within the molecules. Some of the results of this method have been summarized by Robertson, [93] who has contributed largely to this work.

The analysis of x-ray diffraction data for anthracene, naphthalene, and sym.-tetramethylbenzene has led to unambiguous structures for these compounds. Naphthalene and anthracene are composed of plane, regular hexagons in which the carbon-carbon distance is 1.41 Å. This figure is that observed for the C—C distance in graphite. In sym.-tetramethylbenzene the carbons in the ring form a regular, plane hexagon with a C—C distance of 1.41 Å; the methyl carbon atom is 1.47 Å removed from its adjacent ring carbon atom. The methyl groups lie in the plane of the ring and are slightly displaced from the positions demanded by the hexagonal ring, the displacement being 3° greater than that for a symmetrical structure.

The structure of benzoquinone is that proposed originally by organic chemists, except that the ring is slightly skew. An interesting point in connection with the benzoquinone structure is that the carbons joined by a double bond are 1.32 Å apart, while the singly bonded carbons are removed 1.50 Å from each other. The carbon-oxygen distance is 1.14 Å.

The results of the analysis of the structure of bibenzyl show that the two benzene rings are of the regular plane hexagon type; they do not lie in one plane but are parallel to each other and are on opposite sides of the two CH_2 groups. The carbon atoms in the CH_2 groups exhibit tetrahedral bond angles. The CH_2 groups are situated at 1.47 Å from the benzene rings and are removed from each other by 1.58 Å.

[92] Müller, *Proc. Roy. Soc. (London)*, **A124**, 317 (1929).

[93] Robertson, *Science Progress*, **32**, 246 (1937).

The structure of stilbene is different from that of bibenzyl, although the over-all dimensions of the two molecules are rather similar. The stilbene molecule is flat, with all the atoms lying in the same plane. Since the benzene rings are joined to the ethylene group by a single bond, they might be expected to rotate freely and to take a configuration like that of bibenzyl. Their failure to do so is interpreted as being due to resonance, the conjugation between the rings and the central C=C linkage freezing the structure into a planar configuration. Further evidence for resonance is found in the fact that the distance from the CH group to the phenyl group is 1.45 Å instead of 1.54 Å, indicating that there is considerable double-bond character to this linkage.

Robertson and Woodward [94] have determined the structure of diphenylacetylene, which has a triple bond. The molecule is both planar and linear, with the C≡C distance equal to 1.19 Å and the C—C "single" bonds between the central carbons and the rings being 1.40 Å. The value for the C≡C distance agrees very well with that in acetylene, 1.20 Å, determined from the analysis of band spectra.

The structure of oxalic acid has been accurately determined; the molecule is planar. The explanation for this is similar to that given for stilbene in that rotation about the C—C bond is restricted because of conjugation between the carboxyl groups. The existence of resonance is confirmed by the length of the C—C bond, which is only 1.43 Å.

The benzene ring in resorcinol is planar, and the OH groups are distant 1.36 Å from the carbon atoms. The arrangement of the resorcinol molecules in the crystal is interesting. The oxygen atoms are directed toward each other in groups of four and are separated by 2.7 Å; this arrangement is stabilized by the formation of hydrogen bonds. A similar arrangement of molecules in which the oxygen atoms are grouped 2.69 Å distant from each other has been found in pentaerythritol.

One of the best examples of the utility of the x-ray method of determining structure is found in the analyses of phthalocyanine and its nickel salts.[95]

Figure 9 is a graphical description of the unsubstituted phthalocyanine molecule. The distances between the central nitrogen atoms 9—13' and 9'—13 is 2.65 Å; this shortening, as compared with the N—N distance of 2.76 Å between atoms 9'—13' and 9—13, is due to N—H—N bond formation. When the central hydrogens are replaced by Ni to form the nickel salt, the distances between the central nitrogens are 2.56 Å and 2.60 Å, respectively. All the nitrogen and carbon atoms, and probably the hydrogens, lie in one plane. In the central sixteen-

[94] Robertson and Woodward, *Proc. Roy. Soc. (London)*, **A164**, 436 (1938).
[95] Robertson, *J. Chem. Soc.*, 1195 (1936); Robertson and Woodward, *ibid.*, 219 (1937).

membered ring of alternate carbon and nitrogen atoms, the interatomic distance has a practically constant value of about 1.34 Å, and the structure is one of single bond-double bond resonance similar to that found in the benzene rings. Robertson attributes the remarkable stability of the molecule to this inner structure and resonance.
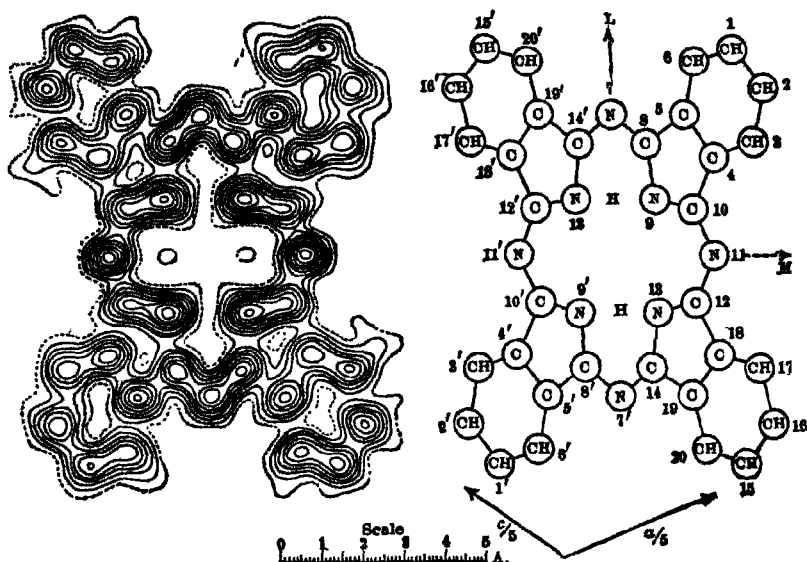
FIG. 9.—Projection along the *b*-axis, showing one complete phthalocyanine molecule. The plane of the molecule is steeply inclined to the plane of the projection, the *M* direction making an angle of 46° with the *b*-axis, and the *L* direction 2.3°. Each contour line represents a density increment of one electron per Å², the one-electron line being dotted.

(Reprinted by permission of the Chemical Society.)

Robertson [96] has determined the structures of the *cis*- and *trans*-azobenzenes. The molecule of *trans*-azobenzene is planar, the N==N distance being 1.23 Å and the N—C distances 1.41 Å; the second value is appreciably less than the expected 1.47 Å and indicates that resonance plays an important part in determining the structure and stability of this form of the compound. The structure of *cis*-azobenzene is not obtained by rotating the N==N bond through 180°; this rotation places the two nuclei 1.3 Å from each other and is not probable from steric considerations. In the most probable configuration, the benzene rings are rotated 40° so that they are separated by 3.1 Å; the N==N distance is 1.23 Å and the C—N distance 1.45 Å; the N—N—C angle is slightly

[96] Robertson, *ibid.*, 232 (1939).

greater than the value 121° observed in the *trans*-form. Resonance appears to contribute little to the stability of this form of the molecule.

The structures of a number of other aromatic compounds have been less accurately determined. All these structures are consistent with those found for the more carefully investigated compounds.

In Table XXV are collected some of the interatomic distances determined from x-ray diffraction data.

TABLE XXV

INTERATOMIC DISTANCES IN SOME ORGANIC COMPOUNDS

Compound	Distance in Å		Compound	Distance in Å	
Diamond	C—C	1.54	Hexamethylbenzene	C—C aromatic	1.42
Graphite	C—C	1.42		C—CH₃	1.48
1,2,3,4,5,6-Hexabromo- cyclohexane	C—Br	1.94	Nonacosane	C—C	1.54
Hexobromoethane	C—Br	1.97	Hydrocarbons	C—C	1.54
1,2,3,4,5,6-Hexachloro- cyclohexane	C—Cl	1.81	Anthracene	C—C	1.41
Hexachloroethane	C—Cl	1.81	Naphthalene	C—C	1.41
Urea	C—N	1.37	Tetramethylbenzene	C—C aromatic	1.41
	C=O	1.25		C—CH₃	1.47
Thiourea	C—N	1.35	Bibenzyl	C—C aromatic	1.41
	C=S	1.64		C—C aliphatic	1.58
Hexamethylenetetra- mine	C—N	1.42		C—CH₂ aromatic- aliphatic	1.47
			Benzoquinone	C—C in ring	1.50
				C=C in ring	1.32
				C=O	1.14
			p-Diphenylbenzene	C—C aromatic	1.42
				C—C between rings	1.48

The study of x-ray diffraction data has proved very valuable in elucidating the gross structures of certain high-molecular-weight compounds.[97] Such data have helped establish the generally accepted-structure of cellulose. In cellulose (p. 1709), it was found that bundles of very long, parallel chain molecules constitute the fibers of ramie, cotton, etc. The chains are composed of unit structures, presumably cellobiose units, which are linked together through oxygen atoms. The length of two units is 10.3 Å, and the distance between two parallel chains is 8.3 Å in one direction and 7.9 Å in the other.

[97] Randall, "The Diffraction of X-rays and Electrons by Amorphous Solids, Liquids, and Gases," John Wiley & Sons, New York (1934), p. 200.

Sisson [98] has considered the data obtained from the diffraction of x-rays by cellulose membranes and has discussed their structure from the micellar and continuous theories. He concluded that there was no necessity to assume a unit smaller than that of a cellulose particle as found in living plants. Mark [99] concluded from the latest available data that cellulose is made up of cellobiose units, which comprise two sets of chains running in opposite directions. The unit cell has the dimensions 8.3Å by 10.3 Å by 7.9 Å.

The structures of certain synthetic linear polymers have been investigated.[100] These compounds appear to have the normal structures of high molecular substances. Whereas, for simple compounds, the arrangement of the end groups determines the crystal form to a considerable extent, for the long-chain substances, these end groups are less important; the simple lattice becomes one in which the ends of the molecules occur at no regular position in the structure. The recent views of such structures picture the molecules as parallel chains of different lengths, with ends overlapping, which in certain regions fit into a lattice-like arrangement. These portions of the structure are termed crystalline regions; in adjacent volumes the chains are in imperfect arrangement and behave as amorphous materials. The polymethylene and polyethylene oxides crystallize with the chains parallel. In the linear polyesters, the ethylene and decamethylene compounds have the planar, zigzag structure, the chain length of which increases uniformly 1.26 Å for each CH_2 group added. The linear polysulfides and vinyl derivatives show similar structures.

The structure of rubber has been the object of much experimental work. The results have been conveniently summarized by Gehman.[101] Stretched and unstretched rubber show different diffraction patterns. Unstretched, raw rubber gives an amorphous pattern like that of a liquid until the substance is frozen, when the pattern becomes crystalline. The change between the two states is continuous. The pattern of frozen rubber is like that of a crystalline organic compound dispersed in minute crystals in random arrangement. On stretching, the diffraction pattern of rubber changes to the crystalline type, with a definite axis of rotation along the axis of stretch. This pattern appears to be superimposed upon an amorphous pattern, and as stretching proceeds the crystalline diffraction increases in intensity. The lattice spacings for stretched rubber are the same as those found for frozen rubber. The interpretations of the x-ray data for rubber are not definitely

[98] Sisson, *Chem. Rev.*, **26**, 187 (1940).
[99] Mark, *ibid.*, **26**, 169 (1940).
[100] Fuller, *ibid.*, **26**, 143 (1940).
[101] Gehman, *ibid.*, **26**, 203 (1940).

settled. The chain molecules in crystallites of stretched rubber are not planar. Current theories postulate a statistical lengthening of the molecules during the stretching of rubber, this lengthening being accompanied by a straightening of the chains. The gross structure can be described by the theory of regions of crystallites and of amorphous volumes. The stereochemically possible structures picture rubber as the cis-butadiene derivative and gutta-percha as the trans-form.

Collagen and gelatin have been investigated by a number of workers, and Astbury [102] has given the most recently proposed structures. These structures are consistent with the chemical data on the degradation products of the proteins and are compatible with their physical properties. In collagen, the long individual molecular chains are held together in the form of a grid by cross linkages of one sort or other between the side chains. The average distance between chains varies with the humidity, but is 10.4 Å for thoroughly dried collagen. The distance between adjacent grids is approximately 4.4 Å. The chains, modified slightly by hydrolysis during the transition from collagen to gelatin, are made up of amino-acid residues, the average length of which along the molecular axis is 2.9 Å. The best evidence indicates that the average chain is 838 Å long or a multiple thereof, and is made up of 288 amino-acid units. The average molecular weight is about 27,000 or some multiple of this. To account for the short average amino-acid length along the direction of the chain, it is necessary to assume a zigzag structure.

The final choice as to the arrangement of the amino-acid residues along the chain of the gelatin molecule is governed by their frequency of occurrence in the decomposition products. The high percentages of proline plus hydroxyproline and of glycine residues lead to the arrangement

$$\cdots-R-G-R-R-G-R-P-G-R-\cdots$$
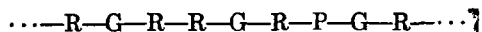
where P stands for either proline or hydroxyproline, G for glycine, and R for one of the other amino-acid residues. This grouping repeats itself until the average molecular weight is 27,000 or a multiple thereof.

ELECTRON DIFFRACTION BY ORGANIC COMPOUNDS

Moving electrons exhibit properties characteristic of wave motion, and the reflection or diffraction of electrons by solids, liquids, and gases obeys certain laws which apply to the action of light or x-rays. Advantage has been taken of this fact in determining molecular structures by a method similar to that used in x-ray diffraction experiments. There is

[102] Astbury, *J. Intern. Soc. Leather Trades' Chem.*, **24**, 69 (1940).

a fundamental distinction between the action of x-rays and electrons; whereas x-rays interact with the electron cloud which surrounds the nuclei, the electrons interact with the nuclei themselves.

The electron diffraction method of determining the structures of substances in the gaseous state has several advantages over the x-ray method. Where measurements are made on gases, complications due to the symmetry properties of intermolecular orientation are lacking and the data are generally more unambiguously interpreted. Such data are more readily obtained by electron diffraction than by x-ray diffraction measurements. Furthermore, the electron diffraction method is capable of showing the locations of hydrogen atoms which cannot be placed by x-ray data; this is of great importance to the organic chemist.

Most of the structures of organic compounds derived from electron diffraction experiments have been determined in the gaseous state.

The determination of molecular structures by electron diffraction measurements was first announced by Wierl.[103] Since this work, many refinements of methods and interpretation of data have been made and a large number of organic compounds have been studied. The results of these investigations have been summarized by Maxwell.[104]

Definite evidence for the planar structure of benzene and the puckered ring structure of cyclohexane has been obtained from electron diffraction data. The structures of a few compounds have been determined by both x-ray and electron diffraction methods, and the agreement between the results obtained by the two methods is satisfactory. This is shown in Table XXVI.

TABLE XXVI

DISTANCES BETWEEN IODINE ATOMS DETERMINED BY X-RAY AND ELECTRON DIFFRACTION MEASUREMENTS

Substance	Electron Diffraction by Vapor	X-Ray Diffraction by Crystal
Iodine	2.64 Å	2.70 Å
1,4-Diiodobenzene	6.85 Å	6.85 Å
1,3-Diiodobenzene	5.97 Å	5.92 Å

A few of the results of electron diffraction studies of organic compounds are given in Table XXVII, taken from Maxwell.

An interesting result of electron diffraction measurements on the vapor of formic acid has been obtained by Pauling and Brockway.[105]

[103] Wierl, *Ann. Physik.*, **8**, 521 (1931); **13**, 453 (1932).
[104] Maxwell, *J. Optical Soc. Am.*, **30**, 374 (1940).
[105] Pauling and Brockway, *Proc. Natl. Acad. Sci. U. S.*, **20**, 336 (1934).

TABLE XXVII

DISTANCES BETWEEN ATOMS DETERMINED BY ELECTRON
DIFFRACTION MEASUREMENTS

Substance	Interatomic Distance in Å		Remarks
Ethane	C—C	1.55	
	C—H	1.09	
Ethylene	C=C	1.34	Plane structure; C—H angle 110°
Acetylene	C≡C	1.22	
Diacetylene	C—C	1.36	Linear
	C≡C	1.19	
Propane	C—C	1.54	Tetrahedral angle
Cyclopentane	C—C	1 52	Plane pentagon
trans-Dichloroethylene	Cl—Cl	4 27	
cis-Dichloroethylene	Cl—Cl	3.22	
Methyl bromide	C—Br	1.91	
Methyl iodide	C—I	2.28	
Methylene iodide	C—I	2.28	I—C—I angle 125°
	I—I	4.06	
Carbonyl sulfide	C=S	1.56	Linear structure
	C=O	1.16	
Bromoform	Br—Br	3.15	Br—C—Br angle 111°
Phosgene	C=O	1.28	Cl—C—Cl angle 117°
	C—Cl	1.68	
Acetyl chloride	C=O	1.14	C—C—Cl angle 110°
	C—C	1.54	
	C—Cl	1.82	
Methyl azide	N≡N	1 24	C—N—N angle 120°
	N=N	1.10	—N=N≡N linear
	C—N	1.47	
Carbon dioxide	C=O	1.13	Linear structure
Carbon disulfide	C=S	1.54	Linear structure
Carbon tetrachloride	C—Cl	1.76	Tetrahedral structure
Benzene	C—C	1.39	Plane hexagonal structure
	C—H	1.08	
Cyclohexane	C—C	1.53	C—C—C angle 109°
	C—H	1.09	
Hexachlorobenzene	C—C	1.41	Plane structure
	C—Cl	1.70	
	Cl—Cl	3.11	
	(*ortho*)		

Their data show that formic acid dimerizes through hydrogen bonds to give the symmetrical structure
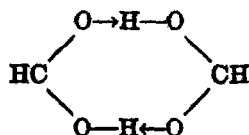
in which the distance between oxygen atoms in the O—H←O system is 2.67 Å and the C—O distance 1.29 Å. The O—C—O angle is 125°. The structure of monomeric formaldehyde has been determined by Stevenson, LuValle, and Schomaker,[106] who found the C—O distance to be 1.21 Å and a C—H distance 1.09 Å.

It has been found that the distances between atoms joined by covalent bonds can be obtained with reasonable accuracy by adding the empirically determined radii of the atoms.[107]

Pauling and Huggins [108] have prepared a set of atomic radii to be used in calculating covalent bond distances. A few of their values are given in Table XXVIII. By the addition of the atomic radius values, the interatomic distances in a number of compounds have been calculated and found to agree with observed values.

TABLE XXVIII

ATOMIC RADII FOR CALCULATING COVALENT BOND DISTANCES

Single Bonds		Double Bonds	
H	0.29 Å	C	0.67 Å
C	0.77	N	0.63
N	0.70	O	0.59
O	0.66	S	0.94
F	0.64		
Cl	0.99		
Br	1.14		
I	1.33	Triple Bonds	
Si	1.17	C	0.61 Å
P	1.10	N	0.55
S	1.04		
Ge	1.22		
As	1.21		
Se	1.17		
Sn	1.40		
Sb	1.41		
Te	1.37		

[106] Stevenson, LuValle, and Schomaker, J. Am. Chem. Soc., 61, 2508 (1939).
[107] Huggins, Phys. Rev., 28, 1086 (1926).
[108] Pauling and Huggins, Z. Krist., 87, 205 (1934).

An example of this agreement is shown in a study by Brockway and Jenkins [109] of the structures of some metallic and non-metallic alkyl derivatives as determined by electron diffraction experiments. In Table XXIX are presented the results of their determinations together with the values calculated assuming additivity of atomic radii.

TABLE XXIX

BOND DISTANCES AND RADIUS SUMS IN METHYL COMPOUNDS

Bond	Experimental Value	Radius Sum	Bond	Experimental Value	Radius Sum
C—C	1.55	1.54	S—C	1.82	1.81
N—C	1.47	1.47	Cl—C	1.77	1.76
O—C	1.42	1.43	Ge—C	1.98	1.99
F—C	1.42	1.41	Br—C	1.91	1.91
Si—C	1.93	1.94	Sn—C	2.18	2.17

The excellent agreement for molecules containing covalent bonds between the observed interatomic distances and those derived from the radius sums has been used as a basis for calculating the degree of resonance in molecules with conjugated systems.[110] For some molecules, the carbon-carbon distance is intermediate between that for a single bond, 1.54 Å, and that for a double bond, 1.32 Å. It is deduced from this that the bond has some single- and some double-bond character, which is the result of resonance between the several possible structures. For example, benzene resonates between the two identical Kekulé structures, and it has been found by electron diffraction measurements that the C—C distances are all 1.39 Å.

By noting the deviations between the experimentally determined and the calculated C—C distances, Pauling, Springall, and Palmer [111] have determined the amount of double-bond character for C—C bonds in various conjugated systems. They conclude that: a bond between two double bonds, two benzene rings, or a double bond and a benzene ring has about 20 to 25 per cent double-bond character; a bond between a double bond and a triple bond has about 30 per cent double-bond character; and a bond between two triple bonds has about 40 per cent double-bond character.

As a result of structure determinations by the electron diffraction method, Schomaker and Pauling [112] conclude that the degree of resonance stabilization of furan, pyrrole, and thiophene increases in the

[109] Brockway and Jenkins, J. Am. Chem. Soc., 58, 2036 (1936).
[110] Pauling, Brockway, and Beach, ibid., 57, 2705 (1935).
[111] Pauling, Springall, and Palmer, ibid., 61, 936 (1939).
[112] Pauling and Schomaker, ibid., 61, 1780 (1939).

order given. From the observed C—N distances in pyridine and pyrazine, 1.37 Å and 1.35 Å, respectively, it is concluded that resonance due to the Kekulé structures is augmented by additional resonance due to ionic structures involving the nitrogen atoms.

ABSORPTION SPECTRUM AND RAMAN EFFECT

It will be profitable to discuss briefly the principles which govern the absorption of light by molecules. A simple diatomic molecule may be considered to have a dumbbell structure in which the two atoms vibrate very rapidly with respect to each other along the line which separates them. In addition to this rapid vibration, the molecule slowly rotates as a whole about its center of gravity. The electronic structure of the molecule in its normal, unexcited state remains constant during the vibration and rotation. When energy in the form of light is absorbed by the molecule, one or all of three changes may occur: the electronic structure of the molecule may be altered; the amplitude of vibration of the atoms may be increased; or the frequency of rotation of the molecule may undergo a change.

When the light absorbed by a molecule results in a change in its electronic structure, the molecule is said to be electronically excited; when the absorbed light causes a change in the amplitude of vibration of the atoms, the molecule is vibrationally excited; when the absorbed light changes the rate of rotation of the molecule, the molecule is rotationally excited. Usually the absorption of light by a molecule results in simultaneous changes in its electronic, vibrational, and rotational states; and in the analysis of the spectral absorption data it is necessary to separate the absorbed light into the three components which contribute to the three types of excitation.

Changes in the electronic structures of molecules require relatively large amounts of energy as compared with those necessary to produce vibrational and rotational excitations. The energy of electromagnetic waves is given by the equation e.v. $= 12,336/\lambda$, where e.v. is the number of electron volts (1 electron volt $= 23,070$ cal.) and λ is the wavelength in Ångström units. The energy necessary for electronic excitation corresponds to light in the ultra-violet or visible region of the spectrum; that for vibrational excitation, to near infra-red light, and that for rotational excitation, to far infra-red light. Consequently, absorption of visible or ultra-violet light usually results in electronic excitation, which is accompanied by rotational and vibrational changes. With the absorption of infra-red light, simultaneous vibrational and rotational excitations usually take place.

In the Raman effect, light of a given wavelength interacts with a molecule and a certain fraction of its energy is used in producing a purely vibrational excitation. The energy not used in this excitation is scattered from the molecule as light of a wavelength longer than that of the exciting source. The difference in wavelengths (or frequencies) of the original and scattered light is therefore a measure of the excitation energy of the vibrating system. Since the Raman effect is measured by differences, the result obtained is independent (within certain limits) of the frequency of the exciting light. Furthermore, since infra-red absorption data also serve as a measure of vibrational energy effects, data obtained from the Raman effect and from infra-red absorption should be comparable; this is found to be true for a number of substances.

The preceding discussion has been confined to diatomic molecules. The problem of the analysis of spectroscopic data for polyatomic molecules becomes exceedingly complicated owing to the large number of vibrating systems and to the increased possibilities for electronic transitions. Indeed, only for polyatomic molecules with high degrees of symmetry have the data been successfully analyzed.

The electronic excitations are more or less dependent on the molecule as a whole. However, the frequencies of the vibrating systems of atoms and groups are more constant, and it has been possible to assign certain characteristic frequencies to the various groups of atoms which occur in organic compounds. It is recognized that constitutional factors have definite effects on the frequencies which the various vibrating atomic couplets exhibit, but the variations due to constitution are usually not too great to impair seriously the assignment of the fundamental frequencies to particular pairs of atoms. Thus, infra-red absorption and Raman-effect measurements are readily interpreted because they do not involve electronic changes, and these measurements have been extensively undertaken in the determination of characteristic group vibration frequencies.

Raman Effect. Since the study of the Raman effect affords a direct, theoretically simple way of determining the fundamental frequencies of vibration characteristic of various atomic linkages, considerable experimental work has been done in the measurement of the Raman effect in organic molecules. Hibben [113] has given an exhaustive summary of the experimental data.

In presenting data on the Raman effect, the usual method of expressing the magnitude of the difference in frequencies of the exciting and emitted light is in terms of reciprocal centimeters, cm.$^{-1}$, i.e., the num-

[113] Hibben, *Chem. Rev.*, **18**, 1 (1936); Hibben, "The Raman Effect and Its Chemical Applications," Reinhold Publishing Corp., New York (1939).

ber of waves per centimeter. The values of these differences are termed the Raman shifts. By using the frequency equation for an harmonic oscillator,

$$\Delta \bar{\nu} = \sqrt{\frac{k}{m}}$$

it is possible to calculate from these Raman shifts the forces binding the atoms of the vibrating system. Force constants for some bonds will be found in Table XXXI.

The use of Raman data is valuable in the identification of certain linkages in organic compounds. The fact that bonds and atomic couplets exhibit characteristic Raman shifts which are independent of the environment of the compounds containing them and, hence, which are free of intermolecular disturbances makes the method of practical importance for structure determination. Constitutive factors affect the absolute values of the shift characteristic of any group, but usually the perturbations are not great enough to prevent identification of the particular bond or group producing the shift. The effect of constitution on the Raman shift for the C—H linkage is shown in Table XXX.

TABLE XXX

RAMAN SHIFTS FOR C—H VIBRATIONS

Linkage		Linkage	
C—H aliphatic	2918	C—H aromatic	3054
H		H	
C—C—H	{ 2930 / 2862	C—C—C	2970
H		H	

In Table XXXI are collected values of the Raman shifts for a few atomic linkages; force constants (k) for some of the bonds are included in the table. The figures represent either the ranges of values observed or rough averages. The force constants for the bonds other than those involving sulfur are relatively independent of the atoms held together, and the bond strengths are in the approximate ratio $1 : 2 : 3$ for the single, double, and triple bonds, respectively.

Attempts have been made by means of Raman spectra studies to determine whether there is unrestricted rotation about a single C—C bond. The data for aliphatic hydrocarbons and saturated aliphatic chlorides are inconclusive, although the results are not incompatible with the existence of cis and trans configurations.

The course of certain polymerization reactions involving opening of

C==C bonds has been followed by noting the behavior of the C==C Raman shift.

TABLE XXXI

RAMAN SHIFTS AND FORCE CONSTANTS FOR CHARACTERISTIC LINKAGES

Linkage	Shift, cm.$^{-1}$	$F \times 10^5$ Dynes cm.$^{-1}$
C—H aliphatic	2910–2970	4.9
C—H aromatic	3050	5.0
C—C aromatic	1580–1620	
C—C aliphatic	990	4.6
C—O alcohol	820–880	5.0
O—H	3500	6.8
C—C ∨ C	1190	
S—H	2570	3.8
C—S	645	2.1
C—N nitro	910–930	4.3
amine	880	
N—O	1000–1080	
C—Cl	650–710	
C—Br	570–600	
C—I	500–530	10.6
C==C	1600–1650	
C==O acid	1650	
ketone	1710	
aldehyde	1720	10–11.5
ester	1720	
anhydride	1750	
N==O nitrate	1640	
nitrite	1640	
nitro	1565	
C==N	1650	10.4
C≡C	2100–2250	15.8
C≡N	2150	17.5

The value of Raman-effect data in determining structures or in differentiating between proposed structures can be illustrated by several of the many problems investigated.

It has been found that the Raman shift due to the C==C bond in *cis-trans* isomers is uniformly greater by at least 15 units for the *trans* compounds than for the *cis* compounds; the importance of this generalization is obvious. By a careful study of the effect on the characteristic C==C shift of substitution on the carbons of the ethylenic linkage, the structures of rhodinol and citronellal have been determined.

It has been found that the characteristic C==O shift of formaldehyde

disappears when this compound is added to water; on solution the Raman spectrum becomes similar to that of glycol. These facts are interpreted to mean that the reaction $CH_2O + H_2O \rightarrow CH_2(OH)_2$ occurs. That no $C=O$ shifts are found in paraldehyde or paraformaldehyde is positive evidence that these polymers are cyclic in structure.

In an equilibrium mixture of a tautomeric substance such as aceto-acetic ester, both $C=C$ and $C=O$ shifts are observed. When the possibility of tautomerism is removed by dialkylation of the central carbon atom, the $C=C$ shift is no longer observable. These facts afford confirmatory evidence for the classical structures of these compounds proposed by the organic chemist.

The presence in the oximes of a Raman shift which corresponds to that of the $C=N$ linkage must be definitely considered in any proposal of a structure for these compounds. Similarly, it has been established from measurements of the Raman effect in nitriles and isonitriles that these compounds contain a $C\equiv N$ linkage.

Infra-red Absorption Spectra. The investigation of infra-red absorption spectra became of particular interest to the organic chemist in 1935, when the results of studies [114] of the infra-red absorption of certain organic molecules containing OH groups were reported. This work immediately opened the way for the extensive investigation by Errera, Wulf, Sutherland, Rodebush, Badger, and others of *chelation* and intermolecular *hydrogen bond* formation in organic compounds. [115]

An organic compound, aliphatic or aromatic, containing a free OH group shows a narrow symmetrical absorption band with its maximum at, or near, 2.7 μ (7000 cm.$^{-1}$); the exact location of the band depends upon the molecule. Hilbert et al. [115a] observed that this band was absent in those molecules which, from other evidence, were known to undergo hydrogen bond formation. It was later established that upon association the narrow band was replaced by a broad one extending from 2.9 μ to 3.3 μ, which was characteristic of the $O—H \cdots O$ linkage.

The change in absorption characteristics of the OH bond of methyl alcohol in carbon tetrachloride solution is shown in Fig. 10, taken from the work of Buswell, Deitz, and Rodebush. [116]

In dilute solutions (0.005 M), the 2.7-μ band of the unperturbed OH group is quite strong, and the broader band due to the $O—H \cdots O$ bond at 2.95 μ is relatively weak. As the concentration of alcohol in-

[114] Wulf and Liddel, *J. Am. Chem. Soc.*, **57**, 1464 (1935); Errera and Mollet, *J. phys. radium*, **6**, 281 (1935); Bloch and Errera, *ibid.*, **6**, 154 (1935).

[115] (a) Hilbert, Wulf, Hendricks, and Liddel, *J. Am. Chem. Soc.*, **58**, 548 (1936); (b) Sutherland, *Ann. Repts. Chem. Soc.* (*London*), **35**, 38 (1938); (c) Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, New York (1939), p. 296.

[116] Buswell, Deitz, and Rodebush, *J. Chem. Phys.*, **5**, 501 (1937).

creases, the number of associated molecules becomes greater and the 2.7-μ band weakens while the 2.95-μ band becomes stronger. In this case, the degree of association is not known, but hydrogen bonding is the cause.
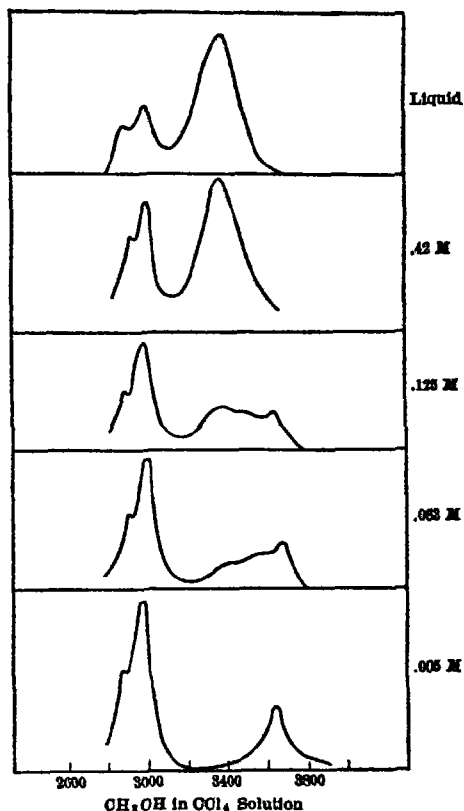
Fig. 10.—Absorption curves for methyl alcohol at various concentrations in carbon tetrachloride and (top) pure liquid methyl alcohol.

Studies [117] of the aliphatic carboxylic acids have shown that, under conditions favoring association, the OH band decreases in intensity and a band characteristic of the dimer appears. Equilibrium constants for the association were calculated from the data and found to agree with those obtained from vapor-density measurements.

Studies have also been made of hydrogen bond formation involving the N—H $\cdots$ O linkage. Buswell, Rodebush, and Roy [118] have investigated the infra-red absorption spectra of acid amides and their mono-

[117] Badger and Bauer, ibid., 5, 605 (1937).
[118] Buswell, Rodebush, and Roy, J. Am. Chem. Soc., 60, 2444 (1938).

and disubstituted alkyl derivatives and of symmetrical ketoximes. Propionamide in carbon tetrachloride solution showed no OH band at 2.7 μ,

$$\overset{\displaystyle OH}{\underset{\displaystyle |}{}}$$

and hence does not enolize to give the structure C_2H_5C═NR. As the concentration of amide was increased, the characteristic NH bands at 2.83 and 2.92 μ decreased in intensity and an O ··· H—N band at 3.15 μ appeared. This change indicates that the molecules associate through hydrogen bond formation. N-Ethylacetamide in dilute solution showed a strong OH band, proving that this compound enolizes. There was a slight shift in the NH band with concentration, indicating that some association was occurring. Disubstituted acid amides gave no spectroscopic evidence for enolization or association.

Symmetrical ketoximes were found to exhibit the strong OH band expected for the structure R_2C═N—OH. With increasing concentration of compound, an association band at 3 μ appeared.

Buswell, Downing, and Rodebush [119] were unable to obtain proof for the formation of N—H ··· N bonds with nitrogen compounds containing no oxygen.

Gordy and Stanford [120] have investigated the infra-red spectra of some compounds containing SH, NH, and NH_2 groups. Their results indicate that the SH group in thiophenol and mercaptans bonds through the hydrogen with pyridine, α-picoline, and dibenzylamine. Pyrrole, diphenylamine, o- and p-toluidine, α-naphthylamine, and o- and m-chloroaniline were found to form N—H ··· O bonds with ether, and to polymerize in the pure state.

The absence of the 2.7-μ band for molecules with OH groups may be due, not to intermolecular hydrogen bond formation (association), but to intramolecular hydrogen bond formation (chelation). Chelation has been extensively studied by infra-red absorption measurements,[115a, 115c] and Table XXXII lists a few of the compounds found to form strong intramolecular hydrogen bonds.

TABLE XXXII

Compounds Undergoing Chelation

o-Nitrophenol	4,6-Diacetylresorcinol
2,6-Dinitrophenol	2,4-Dinitroresorcinol
1-Nitronaphthol-2	4,6-Dinitroresorcinol
2-Nitroresorcinol	2,2'-Dihydroxybenzophenone
Methyl salicylate	1,8-Dihydroxyanthraquinone
o-Hydroxyacetophenone	2,5-Dihydroxydiethylterephthalate
1,4-Dihydroxy-5,8-naphthoquinone	Acetylacetone
1,5-Dihydroxyanthraquinone salicylaldehyde	

[119] Buswell, Downing, and Rodebush, *ibid.*, **61**, 3252 (1939).

[120] Gordy and Stanford, *ibid.*, **62**, 497 (1940).

A study of the molecules found to form intramolecular hydrogen bonds has led to several generalizations regarding the conditions under which the phenomenon occurs.
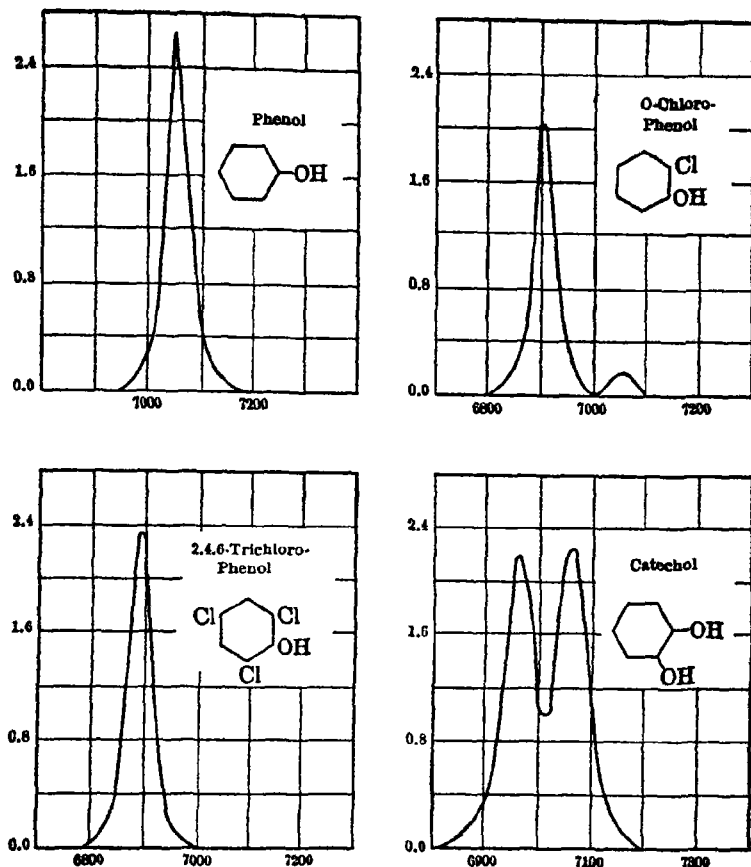
FIG. 11.—Infra-red absorption spectra of phenol and related substances in carbon tetrachloride solution (Wulf and collaborators).

From "Nature of the Chemical Bond," by Linus Pauling, Cornell University Press, Ithaca, N. Y. (1939).

1. The distances between atoms in O—H and N—H groups suffer very little change upon bond formation.

2. The distances of the three-atom system are:

$$O—H \cdots O \qquad 2.6\text{–}2.7 \text{ Å}$$

$$N—H \cdots O \qquad 2.8\text{–}3.0 \text{ Å}$$

3. Chelation takes place only when the ring is free from strain; almost always the ring formed is six-membered, counting the H atom.

4. There must be a limited number of bonds in the ring about which there can be free rotation.

Some hydroxyl-containing compounds show strong absorption in the region 2.7 μ, but the curves do not have the single sharp peak characteristic of aliphatic alcohols. The peaks sometimes occur at wavelengths other than 2.7 μ, and sometimes have two maxima. This is illustrated in Fig. 11, taken from Pauling, by the absorption curves for phenol, o-chlorophenol, trichlorophenol, and catechol.

Pauling [121] explained the double maxima as being due to the existence of two types of OH or NH groups with different frequencies. A phenolic OH group does not interact with other groups *meta* or *para* to it, since resorcinol, hydroquinone, and *m*-nitrophenol show single absorption peaks at 7050 $\pm$ 15 cm.$^{-1}$ Phenol, because of the partial double-bond character of the C—O bond due to resonance, has two possible configurations, but these forms are alike and therefore only a single absorption band is observed at 7050 cm.$^{-1}$ The same geometrical considerations explain the existence of a single band for trichlorophenol, and the frequency maximum at 6890 cm.$^{-1}$ (an appreciable shift from 7050 cm.$^{-1}$) is due to the interaction of the OH with the *ortho* Cl atom.

For o-chlorophenol there are two non-equivalent structures

and
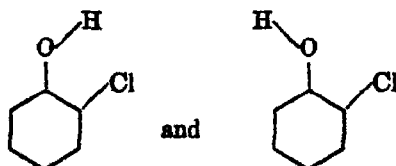
Two molecular species are therefore to be expected, with the *cis*-form in higher concentration because of the stabilizing effect of the O—H $\cdots$ Cl bond. Experimentally, o-chlorophenol exhibits two peaks, one at 7050 cm.$^{-1}$ as in phenol characteristic of the *trans*-form, the other at 6890 cm.$^{-1}$ as in *sym.*-trichlorophenol characteristic of the *cis*-form. The peak at 6890 cm.$^{-1}$ has about ten times the absorption of the other.

The explanation just given for the complexity of the spectrum of o-chlorophenol has been applied to other compounds. Some substances, e.g., o-methoxyphenol, show only one band; this indicates that only one of the isomers of these compounds is stable. Catechol shows two

[121] Pauling, *ibid.*, **58**, 94 (1936).

equal peaks of absorption at 6970 and 7060 cm.$^{-1}$; these are presumably due to the configuration
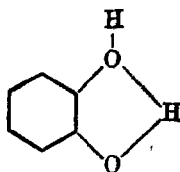
which has two OH bonds of different types in equal amounts.

Davies [122] has studied the infra-red spectrum of chloral hydrate in the range 2.6–3.0 μ and has found a strong band at 2.8 μ which can be due only to the OH bond. He concludes that the anomalous stability of two OH groups on the same carbon atom in chloral hydrate is due to the interaction of the OH groups with the adjacent CCl$_3$ group, i.e., to form O—H $\cdots$ Cl bonds. Bromal hydrate was found to exhibit the same general spectral properties as chloral hydrate.

The use of infra-red absorption measurements in studying the association and chelation of organic compounds affords an excellent example of the utility of a physical measurement which yields direct information about the behavior of definite structural units in complex molecules. Such a method offers distinct advantages over others such as the parachor. This is true because the parachor value represents the sum of all the structural units in the molecule, and hence necessitates that individual groups or units be identified by taking differences, a procedure frequently inaccurate.

Visible and Ultra-Violet Absorption Spectra. As previously stated, the absorption of visible or ultra-violet light is usually associated with a change in the electronic structure of one or several parts of the absorbing molecule, accompanied by alteration in the vibrations of the atoms in the groups and by changes in the molecular rotation. If only electronic excitation occurs, the absorption is confined to a single wavelength, as is found in the spectra of certain rare-earth metals; it is the additional changes in vibration and rotation which are responsible for the broad nature of the observed absorption bands.

It frequently happens that there are two groups in the molecule which absorb light. If these groups are isolated from each other in the sense that there is little or no electronic interaction between them, the absorption spectrum usually exhibits two separate absorption bands, each characteristic of one absorbing group. If the two groups interact electronically, usually through conjugation, the resultant absorption shows a strongly modified band or bands.

[122] Davies, *Trans. Faraday Soc.*, **36**, 333 (1940).

The types of interaction between two groups may be broadly and loosely classified by their effects upon the absorption spectrum of the molecule. The first type of interaction is found when two relatively non-polar groups are joined by conjugated double bonds, and the absorption spectra of such systems are usually in the ultra-violet or near ultra-violet. Although resonance plays a part in determining the spectra of such compounds, its marked effect is shown in the second type of interaction usually found in strongly colored substances, dyes. This was first pointed out by Bury.[133]

Lewis and Calvin [134] in an excellent review of the absorption behavior of organic substances have critically examined the pertinent data and have formulated a general theory about the color of compounds. They describe resonance as follows:

"A substance to which only one reasonable formula can be assigned has properties in accord with that formula. When to a substance two or more structures might be assigned, such that one may be derived from another merely by the shifting of electron pairs, these structures are said to be in resonance with one another, especially if the energies corresponding to the several structures, as well as the relative positions of the atoms, are not widely different. Such a resonating substance is to be regarded not as a mixture of various molecules corresponding to the various structures, but rather as having a single kind of molecule which, however, partakes of the character of the several contributing structures." Resonance is usually responsible for the deep color of certain organic compounds. The individual groups making up the molecules have their characteristic absorptions in the ultra-violet or near ultra-violet, and resonance causes the absorption in the visible region.

Most colored organic compounds are only slightly volatile and have been studied only in solution. In solution, the rotational fine structure is completely wiped out, and that due to vibrational effects is usually not resolved. This results in a broad band which may or may not show minor peaks. It is sometimes possible to resolve the vibrational structure by observing the spectrum at low temperatures, where the perturbing effects of the solvent molecules are considerably diminished. This is illustrated in Fig. 12, taken from Lewis and Calvin.

The solvent used may have a marked effect upon the absorption spectrum, particularly when there is reaction such as salt formation between the solute and the solvent or when solvation occurs, frequently through hydrogen bond formation. This effect is shown in Fig. 13 for

[133] Bury, *J. Am. Chem. Soc.*, **57**, 2115 (1935).
[134] Lewis and Calvin, *Chem. Rev.*, **25**, 273 (1939); Branch and Calvin, "The Theory of Organic Chemistry," Prentice-Hall, New York (1941).
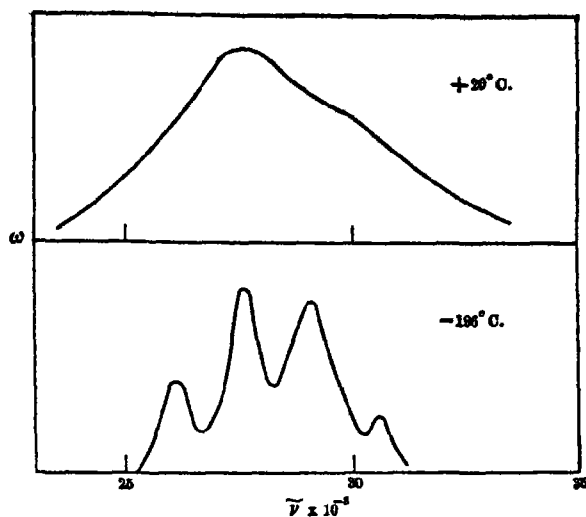
FIG. 12.—Absorption curves of dodecapentaenic acid in a mixture of ether and alcohol.
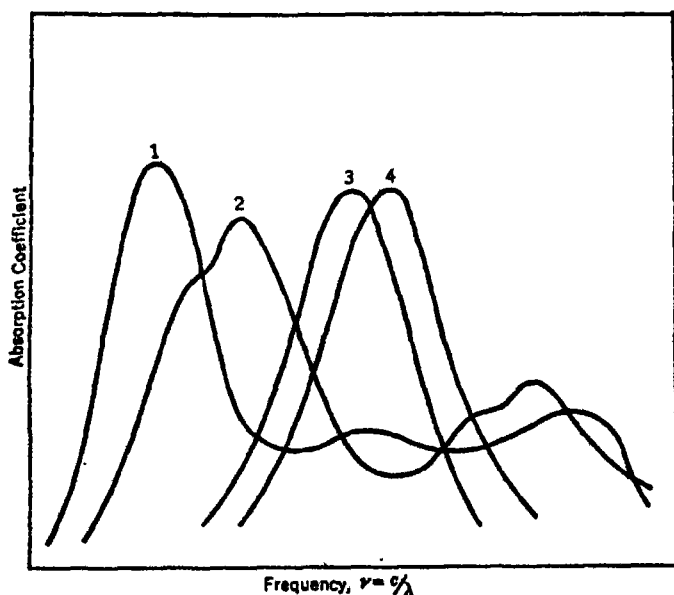
FIG. 13.—Influence of solvent on the absorption spectrum of benseneazophenol.
1 = HCl solution; 2 = pyridine solution; 3 = alcohol solution; 4 = KOH solution.
From data of Brode, loc. cit.   (Courtesy of publishers.)

benzeneazophenol, taken from Brode.[125] In order to reduce solvent effects, measurements are made in non-polar or slightly polar media, if solubility relations permit it.

It will not be possible to develop fully or to illustrate amply the various ramifications of theories of color. For a more detailed treatment, reference should be made to the paper of Lewis and Calvin and to leading citations given therein. For the present purpose, it will be possible to give only a few illustrations of the absorption behavior of various types of organic compounds.

The effect upon absorption of the interaction of two relatively non-polar groups which exhibit resonance to a minor degree is illustrated by observations on the absorption spectra of solutions of aldehydes.[126] The spectra of benzene, propionaldehyde, benzaldehyde, phenylacetaldehyde, and hydrocinnamaldehyde were investigated. The introduction of the aldehyde group into benzene profoundly modified the absorption of benzene. The separation of the CHO group from the benzene ring by a CH_2 group as found in phenylacetaldehyde resulted in less modification of the benzene absorption, and the introduction of two CH_2 groups resulted in a spectrum in which the absorption due to the ring was practically identical with that of benzene. Simultaneous with the above changes, there were alterations in the absorption of the CHO group as found in propionaldehyde; in hydrocinnamaldehyde the carbonyl absorption was identical with that of propionaldehyde. The absorption spectrum of hydrocinnamaldehyde was the same as that of an equimolal mixture of benzene and propionaldehyde. It appears from these data that interaction effects are completely eliminated between the carbonyl and phenyl groups when these groups are separated by two methylenes.

Smakula [127] has summarized the results of an extended investigation by Hausser, Kuhn, et al. of the absorption characteristics of compounds containing conjugated chains. The compounds studied did not show large resonance effects because of their structures. The classic example of the effect of increasing the length of the conjugated chain is given in Fig. 14 for the diphenylpolyenes. In the series, the number of double bonds was varied continuously from one to seven.

The positions of the absorption maxima move progressively to longer wavelengths as the number of double bonds in the molecule increases. Lewis and Calvin have found a linear relationship between the square of the wavelengths of maximum absorption and the number of double

[125] Brode, J. Phys. Chem., **30**, 56 (1926).
[126] Arnold and Kistiakowsky, J. Am. Chem. Soc., **54**, 1713 (1932).
[127] Smakula, Angew. Chem., **47**, 657 (1934).

bonds in the chain.   Their plot for this is given in Fig. 15.   They ex-
plain this linearity by assuming that the vibrating C==C groups, al-
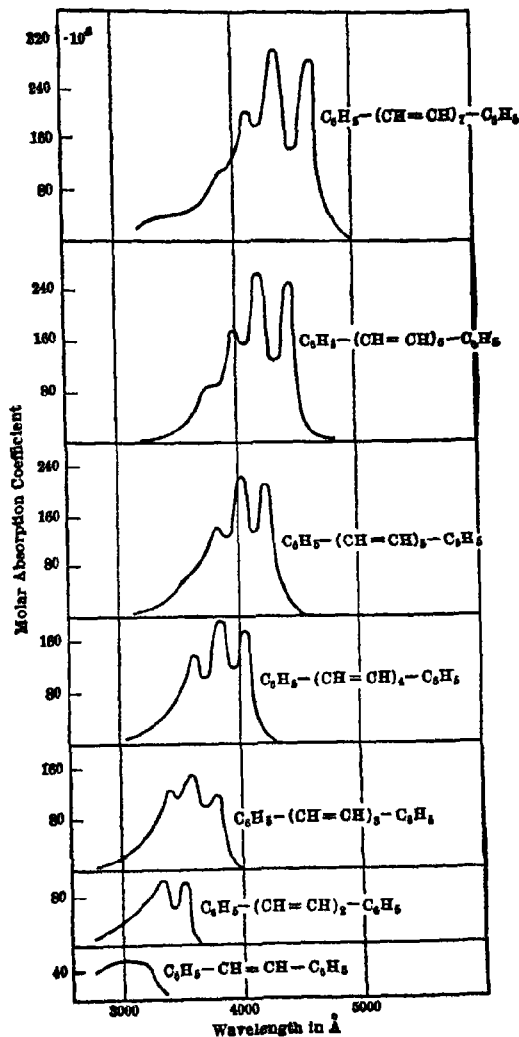
FIG. 14.—Absorption spectra of diphenylpolyenes in benzene.

though retaining their identity in the chain, interact to behave as a single
oscillator which obeys the equation of an harmonic oscillator.

The molecular absorption coefficient, ϵ, is defined by $I/I_0 = 10^{-\epsilon c d}$,
where I_0 is the incident and I the transmitted light, c is the molar

concentration, and d the length of path in the absorbing medium. For the polyenes, the coefficients depend on the number of double bonds, each bond behaving as though independent of the others; the ϵ of a compound is therefore about equal to that for the homolog with one double bond multiplied by the number of double bonds. This is shown in Fig. 14.

Witt [128] formulated the first theory of color. He stated that a dye must have one or more groups called chromophores, which are un-
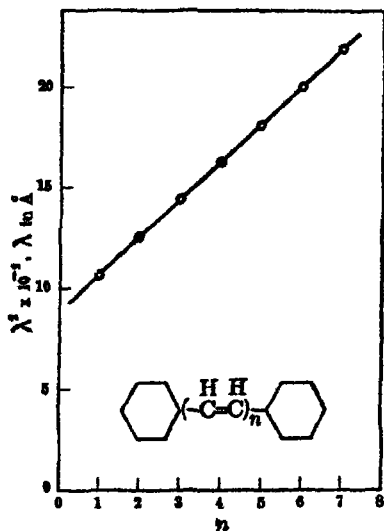
Fig. 15.—Plot of the square of the wavelength against the number of units of the polyenic chain.

saturated groups showing absorption in the ultra-violet or near ultra-violet. Examples of well-known chromophores are $C=C$, $N=N$, $C=O$,

$N=O$, and combinations of these linkages, e.g., $N=O$. In simple molecules it has been possible to assign approximate absorption regions to the various chromophoric groups; for example, in aliphatic ketones and aldehydes the carbonyl group absorbs in the region 2600–3000 Å, and the nitro group in nitromethane absorbs at about 3600 Å. In addition to the chromophores, a molecule might have other groups called auxochromes, which, though showing no absorption in the visible part of the spectrum, have the power of greatly enhancing the color of a chromophore-bearing substance. It is now recognized that the function of the

[128] Witt, Ber., 9, 522 (1876); 21, 321 (1888).

auxochromes is to increase the resonance of molecules; examples of such groups are OH and NH_2.

Bury applied the theory of resonance to the fact that certain compounds show much deeper color than would be expected from their chromophores. As an example he cites Döbner's violet, which exhibits a high degree of resonance because of the existence of two equivalent structures
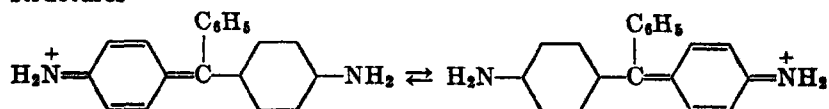
This compound therefore has a much deeper color than would be expected from the non-chromophoric NH_2 group and the three phenyl groups which absorb in the ultra-violet. The compound p-aminotriphenylmethane hydrochloride is colorless, and examination will show that no resonating structures of equal energy are possible. The introduction of the non-chromophoric NH_2 group, which is an auxochrome in Witt's terminology, into one of the unsubstituted phenyls introduces resonance, and the deep violet color of the dye results. Similarly, the introduction of the non-chromophoric OH group into the colorless sodium p-hydroxytriphenylmethane promotes resonance between the structures
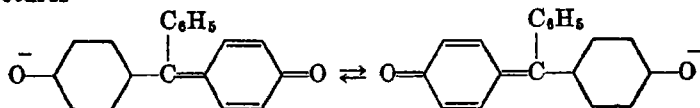
and the resulting product, benzaurin, is a dye.

The cyanine dyes, extensively studied by Brooker and co-workers [129] and by Fisher, Hamer, et al.,[130] are intensely colored substances exhibiting a high degree of resonance. These dyes have the general structure
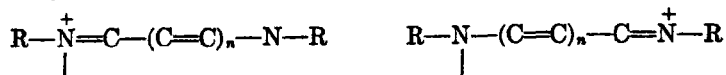
Lewis and Calvin have plotted the absorption maxima for the series of dyes
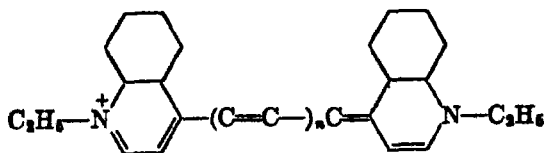
[129] (a) Brooker and Keyes, J. Am. Chem. Soc., 59, 74 (1937); (b) Brooker and Smith, ibid., 59, 67 (1937); (c) Brooker, Sprague, Smyth, and Lewis, ibid., 62, 1116 (1940).

[130] Fisher and Hamer, Proc. Roy. Soc. (London), A154, 703 (1936); Beilenson, Fisher, and Hamer, ibid., A163, 138 (1937).

against the number of double bonds in the chain, as shown in Fig. 16. The linear relationship between the absorption maximum and the number of double bonds in the chain is striking. This is explained by Lewis and Calvin by assuming that the high degree of resonance causes the
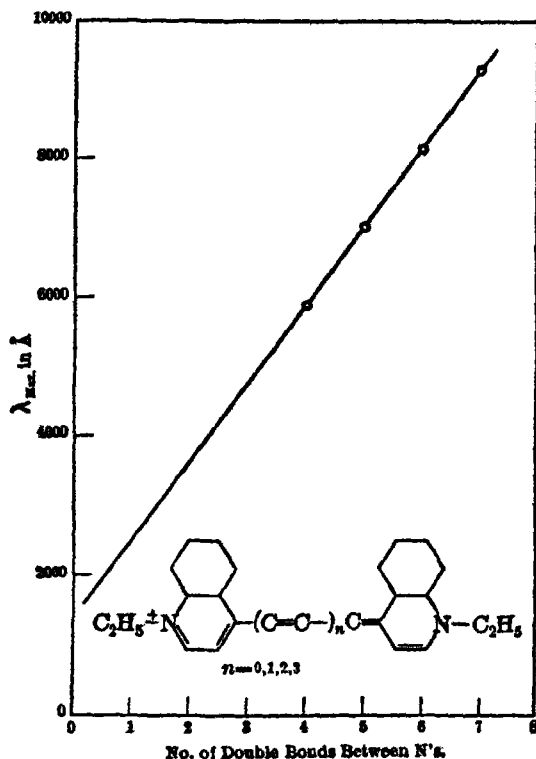
Fig. 16.—Plot of wavelength against the number of double bonds between the two nitrogen atoms for a series of carbocyanines.

electrons in the chain system to be uniformly distributed so that the system behaves as an elastic string, where the vibration frequency is inversely proportional to the length of the string.

A direct attack on the problem of the relationship between resonance and light absorption has been made by Brooker, Sprague, Smyth, and Lewis, [129a] who compared the absorptions of the vinylene homologous series of thiacyanine dyes, I (n = 0, 1, 2), with those of the bases, II (n = 0, 1, 2), which are derived from the dyes. The absorption spectra of the dyes and their bases differ in several important respects. In each case the base absorbs at shorter wavelength than the

I

$C=CH-(CH=CH)_n-C$ ... $N+$ ... Et ... Et ... $I-$

(a)

$C-(CH=CH)_n-CH=C$ ... $N+$ ... Et ... Et ... $I-$

(b)

II

$C=CH-(CH=CH)_n-C$ ... Et

(a)

$C-(CH=CH)_n-CH=C$ ... $N+$ ... Et ... $N-$

(b)
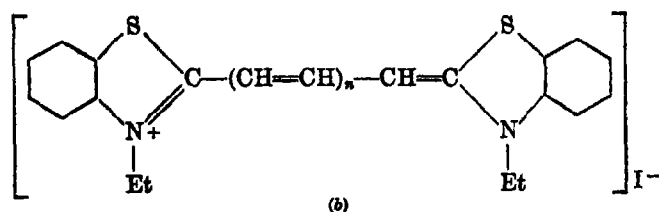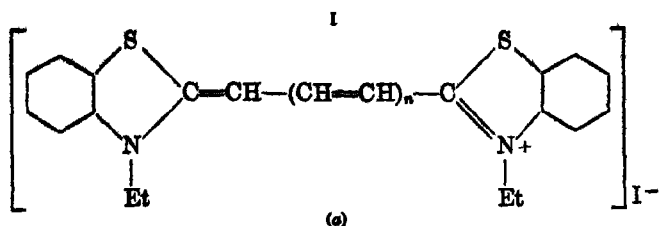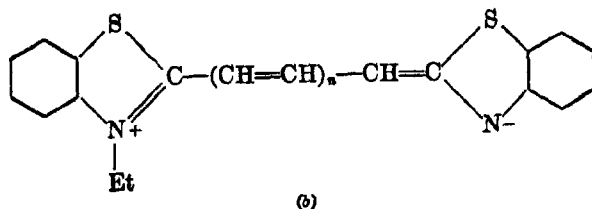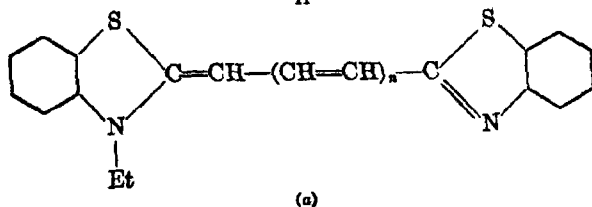
cyanine with the same chain length, and the difference in λ_{max}. between the base and the cyanine increases with the number of conjugated double bonds. In the cyanine series, the value of the molecular absorption coefficient, ϵ_{max}, increases continuously as the series is ascended, but the values of ϵ_{max}. are very close together for the bases and are much lower than those for the corresponding cyanines.

The fact that the absorption spectra of a cyanine and of its base of the same chain length are very different provides a further demonstration that the presence of conjugation is insufficient to define the absorption of a compound.

The cyanines each have two identical resonance configurations, Ia and Ib, giving absorption curves which rise, in the first few members, to a high value of ϵ_{max}, and which are widely separated with respect to

wavelength. On the other hand, it is not possible to devise two identical resonance configurations for the bases II. There is, however, a certain probability for the dipolar formula II*b*, and resonance between II*a* and II*b* has been suggested by Brooker *et al*. In II*b* the quaternary nitrogen atom is normal, but the $>N^-$ in the benzothiazole nucleus is not likely to be very stable, and the actual state of the molecule tends to approach II*a*, with a corresponding loss of resonance. In view of the fundamental assumption concerning resonance and color, the bases II are therefore less deeply colored than the very highly resonating cyanines.

It was further argued by Brooker *et al.* that, if $>N^-$ is a point of instability in II*b*, the stability of the conjugated system should be increased by replacing the benzothiazole nucleus by one in which $>N^-$ is more stable. For this purpose, an indole nucleus was chosen, since indoles with hydrogen attached to nitrogen characteristically yield potassium derivatives, and hence show a strong tendency to form negatively charged indolyl ions in which $>N^-$ is stable.

In direct support of the deductions of these investigators, the base III was found to be actually more deeply colored than its methiodide,

III

(a)

(b)
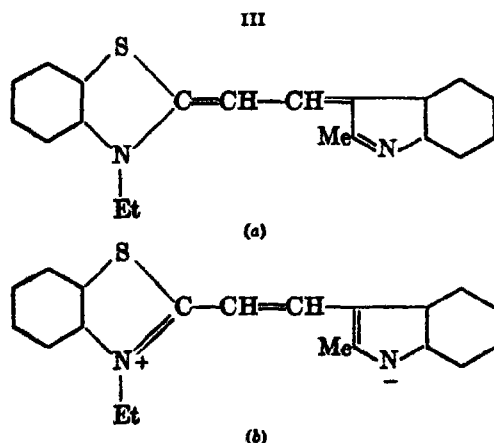
IV. This result was ascribed to a combination of two distinct, although related, factors. The first is that III resonates relatively strongly, owing to the stability of $>N^-$ in III*b*, and the second is that IV is abnormally light in color. This latter fact is explained on the basis that the indole nucleus is less basic than the benzothiazole nucleus and hence that IV*a* is less stable than IV*b*. There is a tendency toward IV*b* accompanied by loss of resonance, and the dye therefore absorbs at shorter wavelengths than would be expected. It was demonstrated by the synthesis of other compounds that the presence of the indole nucleus alone was not sufficient to confer deep color. Confirmation of the argu-

ments was provided by a comparison of the dipole moments. The structures postulated to have dipole modifications were found to have moments definitely greater than those calculated from the classical formulas.

IV

(a)

(b)
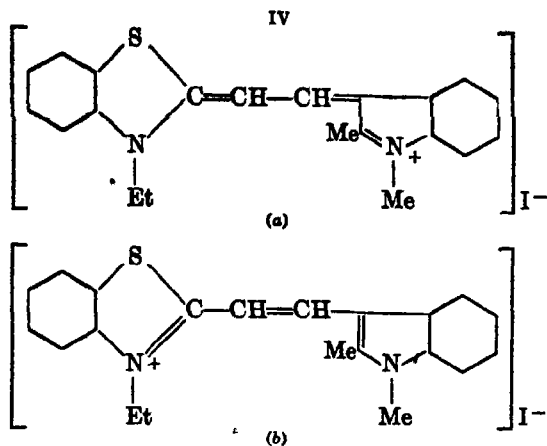
Many additional examples of resonance are known and have been discussed. At the present time there appears to be no strong argument against the general concept as applied to the theory of color, although there have been other approaches to the problem. Burawoy [131] has attempted to classify chromophoric groups and absorption bands into two types. These are the R (radical) chromophores, which are due to double bonds and occur in simple compounds like acetone and nitromethane, and the K (conjugation) chromophores, which are sensitive to polar groups such as NH_2 and OH. Burawoy explains the behavior of these types on the basis of electron isomerism, in which double bonds may open to give *quasi* free radicals. He differentiates between the effect of electron isomerism and resonance, stating that there is no connection between them. Burawoy's facts and arguments do not appear strong enough to merit preference over the resonance approach to the problem.

The intense color of the triarylmethyl free radicals was first investigated by Meyer and Wieland.[132] The color of these compounds cannot be explained on the simple resonance concept. Lewis and Calvin have suggested that the presence of the odd electron in the free radicals produces a very mobile electron cloud which easily interacts with the incident light.

[131] Burawoy, *J. Chem. Soc.*, 1177 (1939).
[132] Meyer and Wieland, *Ber.*, 44, 2557 (1911).

Conrad-Billroth [128] has published a series of papers on the absorption spectra of aromatic compounds and has pointed out certain relationships between the nature of the absorption band and other physical properties such as the dipole moment. Attempts to resolve the absorption spectra of organic compounds into additive and constitutive components have not proved successful. [134] This is undoubtedly due to the as yet unanalyzed contributions of the parts of the molecules to their resonance. Until this analysis is made, it does not appear to be possible to segregate the effects of individual groups upon the integrated spectrum.

The use of absorption spectra has in some cases been of value in studying the structures of compounds. Such studies have been made, particularly by Hantzsch, with acetoacetic ester and have established the existence of its tautomers. Similarly, the spectra of certain derivatives of phloroglucinol have given direct evidence for the hydroxy and keto forms of this compound.

The practical value of absorption measurements is well known to the worker in the field of natural products. When complex compounds are being investigated, the absorption spectrum is frequently one of the few properties used for identification. This is illustrated by studies of pyrrole pigments by Stern, Wenderlein, and Molvig. [125]

THERMODYNAMIC PROPERTIES OF ORGANIC COMPOUNDS

For the organic chemist, the most useful function of thermodynamics is the prediction of the course and extent of chemical reactions. When the required thermal properties of reacting substances and of their products are known, it is possible to calculate to what extent a reaction will proceed. However, the fact that a reaction is energetically possible does not insure that it will go under normal conditions, since thermodynamics can give no information as to its rate. For example, calculations show that the reaction of hydrogen and oxygen to form water will come to equilibrium when the gases are present in less than 1 part in 10^{10}; however, at room temperature the gases do not react at a measurable rate unless they are irradiated with ultra-violet light or are in contact with a suitable catalyst.

The thermodynamic method is empirical in the sense that it is based upon certain arbitrary definitions and upon the experimentally proved infallibility of the so-called first, second, and third laws. By mathe-

[123] Conrad-Billroth, Z. physik. Chem., B25, 139 (1934); B20, 222 (1933).

[134] Wolf and co-workers, ibid., B13, 201 (1931); B21, 389 (1933).

[125] Stern, Wenderlein, and Molvig, ibid., A170, 337 (1934); A174, 81 (1935); A177, 40 (1936).

matical analyses of the defined functions consistent with the observance of the three laws, certain relationships between experimentally determinable quantities are derived. The three most important thermodynamic quantities are the free energy, F, the heat content, H, and the entropy, S.

Though F, H, and S are mathematically defined and are not dependent for definition upon any restricted physical process, they can be measured by certain processes and can be loosely interpreted in terms of such processes. For practical use, the chemist is interested chiefly in the change in free energy, ΔF, which accompanies a reaction; this is obtained by subtracting the sum of the free energy contents, F_3, F_4, etc., of the products from that of the reactants, F_1, F_2, etc.; i.e., $\Delta F = F_1 + F_2 - F_3 - F_4$. The change in heat content, ΔH, and the change in entropy, ΔS, are similarly obtained from the heat contents and entropies of the reactants and products.

The equation used in determining the extent of a reaction is

$$\log K = - \frac{\Delta F}{2.3RT}$$

where R is the gas constant, T the absolute temperature, and K the equilibrium constant. If $K = 1$, the reactants and products of a reaction $A + B \rightleftarrows C + D$ will be in equilibrium when the reaction has proceeded 50 per cent. If $K = 100$, the reaction reaches equilibrium when it is about 90 per cent complete, and if K is 0.01 the reaction will go about 10 per cent to completion. At room temperature, $\log K = -\Delta F/1400$ (approximately); consequently, if ΔF is less than -4200, $\log K$ is greater than 3 and K is greater than 1000. The reaction may therefore be considered to go to completion. If ΔF is greater than $+4200$, the reaction will stop when less than 1 per cent completed, provided that the equilibrium is not disturbed by precipitation, evaporation, etc.

The entropy of a substance may be very loosely considered to be a measure of the randomness of arrangement of its molecules in space. At absolute zero, where all intermolecular forces are zero and the arrangement of molecules is perfect, the entropy is zero. With increasing temperature, the molecules acquire thermal agitation and take on progressively less perfect arrangements, and the entropy rises. In the transition from the solid to the liquid state, there is an abrupt loss of order and the entropy increases markedly; and upon vaporization to give a more or less completely random arrangement of molecules, the entropy increases again. Trouton's rule states that for non-associated liquids the latent heat of vaporization, Q, divided by the absolute tem-

perature is a constant, approximately 23. This is simply a statement that the entropy of vaporization, $S = Q/T$, is constant for such liquids.

The change of heat content, ΔH, accompanying a reaction is equal to the heat of the reaction at constant pressure. This quantity is frequently used in calculating changes in free energy. It is also of importance in calculating the equilibrium constant for a reaction at a given temperature from that found at another temperature. The equation is

$$\frac{d\ln K}{dT} = \frac{\Delta H}{RT^2}$$

The free energy changes for reactions may be calculated from the equation $\Delta F = \Delta H - T\Delta S$, and ΔH and ΔS may be determined from thermochemical data. The heat content, H, for a substance at temperature T is equal to the heat capacity, Cp, measured at constant pressure and integrated from absolute zero to T. The entropy, S, is equal to the quotient Cp/T integrated from zero to T. It is not easy to measure the heat capacities of substances at very low temperatures, but some systematic investigations of organic compounds have been made.

The thermodynamic properties of organic compounds have been discussed in detail in a number of publications. From measurements of the heat capacities of certain organic compounds and of their elements over a wide range of temperatures, Parks and Huffman [136] have calculated entropies and heat contents. These values, in conjunction with measured heats of reaction, have permitted the calculation of the free energies of formation of the compounds from their elements.

Parks and Huffman found that the entropies of the members of certain homologous series, e.g., the n-hydrocarbons, increase linearly as the series is ascended. The free energies of formation, ΔF, do not form a regular series, although variations are in the same direction and of the same order of magnitude. When alkyl groups are substituted for hydrogen in a n-hydrocarbon, the entropy of the branched-chain compound is less than that of the normal compound having the same number of carbon atoms. The formula $S_{298} = 25.0 + 7.7n - 4.5r$, where S_{298} is the entropy at 25° C., n is the number of carbon atoms in the compound, and r is the number of branched chains (alkyl) in the molecule, fits the data for a number of isomeric pentanes, heptanes, and octanes satisfactorily. The heats of formation and free energies of formation at 25° C. vary in like manner with the introduction of side chains into the n-hydrocarbons; ΔH_{298} is lower for the branched-chain

[136] (a) Parks and Huffman, "Free Energies of Some Organic Compounds," Am. Chem. Soc. Monograph Series, No. 60, Chemical Catalog Co., New York (1932); (b) Rossini, J. Research Natl. Bur. Standards, 13, 21, 189 (1934).

compounds, and ΔF_{298} is higher. The heats of formation and free energies of formation of unsaturated hydrocarbons are greater than those of the corresponding saturated compounds; the differences decrease as the unsaturation becomes buried in the molecule.

Three general equations have been given for the entropies of n-hydrocarbons in the liquid, gaseous, and solid states at 25° C.

$$S_{298} \text{ (liquid)} = 25.0 + 7.7n$$

$$S_{298} \text{ (gas)} \quad = 34.0 + 10.0n$$

$$S_{298} \text{ (solid)} \quad = 18.0 + 5.8n$$

In these equations, n is the number of carbon atoms in the molecule. Parks and Huffman have considered organic compounds as derivatives of the hydrocarbons, and have prepared a list of substitution factors which, when added to the entropy values for the parent hydrocarbons, give corresponding entropies for the compounds. Their substitution factors are given in Table XXXIII. In this table substitution factors

TABLE XXXIII

MOLAL ENTROPY AND FREE ENERGY FACTORS

Structural Modifications	Change in Molal Entropy			Change in ΔF_{298}
	Solid	Liquid	Gas	
Addition of CH_2 in chain......... ...	5 8	7.7	10	1,080
Substitution of CH_3 for H (chain)....	5 0	3 2	5.0	1,900
C_2H_5 for H (chain)..	...	10.9	. .	3,000
CH_3 for H (ring)......	5.8	7.7	. ..	....
C_2H_5 for H (ring)... .	11.6	15 4	....	1,100
C_6H_5 for H............	17 0	19 5	....	36,000
Cyclohexyl for H.. ..	....	26.5	....	13,000
Conversion C—C to C=C........... .	−2.7	−2.7	−2.7	20,000
Sub. OH for H (primary alcohol)... .	0	−1.5	13.0	−34,000
(secondary alcohol).....	0.5	−4 0	9.0	−37,000
(tertiary alcohol).	0.5	−6.0	7.0	−41,000
(phenol)...........	0	0	....	−41,000
Sub. of —O— to form ether...... ...	....	5.0	8.0	−20,000
Sub. of =O for 2H (aldehyde)	1.0	5.0	....	−23,000
=O for 2H (ketone).... . ..	1.0	0.5	6.0	−30,000
CO_2H for H (acid).......... . .	5.8	7.7	....	−83,200
NH_2 (amine)...... .	0 0	0.0	....	6,000
NO_2 (nitro compd.).....	7.0	8.0	....	7,000
Cl..........	6.0	7.0	9.0	−1,600
Br........................	7.5	9.0	11.5	4,500
I...........................	9.0	11.0	14.0	10,000

have been given for the solid, liquid, and gaseous states. In comparing thermodynamic data to determine the effect of additive and constitutive factors, more consistent results are to be expected when the data apply to the gaseous state; this is because intermolecular forces are almost completely absent in the gas.

The thermodynamic data for some aliphatic and aromatic hydrocarbons have been summarized by Rossini,[137] Pitzer,[138] Aston,[139] and Parks.[140]

Conant and Thompson [141] have studied the equilibria between enol and keto forms of acetoacetic ester and acetylacetone and some of their derivatives. The equilibria were determined for the gaseous phase in order to eliminate effects due to intermolecular forces, and free energy changes were calculated from the data. The results show that there is a relationship between the structure of a compound and the free energy of enolization in the gaseous state; because of solvent effects, this relationship is obscured when the equilibria are measured in the liquid state. It was found that replacement of a hydrogen of the central carbon atom by a primary alkyl group increased ΔF by 1 kcal., and by a secondary alkyl group by about 1.5 kcal. Substitution of a phenyl group was found to decrease ΔF by 0.7 kcal., thus increasing enolization as contrasted with the effect of other substituents which decreased the tendency to enolize. From the fact that the enol-keto equilibria were not greatly affected by temperature, it may be concluded that ΔH of enolization is small.

The study of the thermodynamic properties of organic compounds has led to values for the free energies, entropies, and heat contents of a number of substances, but the greatest amount of data has been the result of measurements of heats of combustion. These data are expressed as the number of calories evolved or absorbed when an organic compound reacts with oxygen to give CO_2, H_2O, NO_2, etc.

Kharasch [142] has compiled and evaluated the available combustion data on organic compounds and has developed a method of calculating the heats of combustion of liquid substances. His formula is $Q = 26.05n + C$, where Q is the heat of combustion, 26.05 the net amount of energy change per mole electron, n the number of valence electrons, and C a constant characteristic of the electron linkage. The values of the constant C for some linkages are given in Table XXXIV.

[137] Rossini, *Chem. Rev.*, **27**, 1 (1940).

[138] Pitzer, *ibid.*, **27**, 39 (1940).

[139] Aston, *ibid.*, **27**, 59 (1940).

[140] Parks, *ibid.*, **27**, 75 (1940).

[141] Conant and Thompson, *J. Am. Chem. Soc.*, **54**, 4039 (1932).

[142] Kharasch, *Bur. Standards J. Research*, **2**, 359 (1929).

TABLE XXXIV

VALUES OF THE CONSTANT C FOR CALCULATING HEATS OF COMBUSTION

Coupling	C	Coupling	C
Aromatic to aliphatic	−3.5	Aromatic to aromatic	−6.5
Double bond		Triple bond, one H	+46.1
aliphatic, *cis*	16.5	no H	33.1
aliphatic, *trans.*	13.0	Primary alcohol	13.0
aromatic to aliphatic	−6.5	Secondary alcohol	6.5
in ring	+6.5	Tertiary alcohol	3.5
Aldehyde (RCHO)	13.0	Phenol	3.5
Ketone (RCOR)	6.5	Ester (RCO$_2$R)	16.5

When the heats of formation of the products of a reaction are known, the measurement of the heat of reaction provides a means of calculating the heats of formation of the initial substances. In order to make such calculations for organic compounds, it is necessary to know the heats of formation of the carbon compounds in the reaction products. The absolute values of the heats of formation of carbon compounds depend upon the heat of sublimation of carbon, which has not yet been accurately determined. However, by making reasonable assumptions it is possible to construct a set of figures for heats of formation which can later be readily converted to their true values when an accurate figure for the heat of sublimation of carbon has been obtained. Such a set of heats of formation is internally consistent and is reliable for making comparisons between compounds. From such a set it is possible to calculate empirical bond energies, that is, the amounts of energy necessary to break the various chemical bonds.

The energies of a number of bonds in inorganic compounds have been directly determined by thermochemical and spectroscopic methods. Starting with these as a basis, Pauling [148] has calculated the energies of a number of bonds involving carbon. He assumed that the total energy content of a molecule could be determined by adding the energies of its individual bonds. This assumption of additivity of bond energies has proved to be justified as a first approximation.

Rossini [186b] has studied the heats of formation of normal aliphatic hydrocarbons and has discussed the energies of the bonds in these compounds. He points out that there are several types of C—H (and C—C) linkages, depending upon their position in the molecules, and that their energies are not identical. For example, to split the first hydrogen from methane requires a different amount of energy from that required to remove the last. This shows that the bond energies depend not only on

[148] Pauling, *J. Am. Chem. Soc.*, **54**, 3570 (1932).

the nature of the atoms which are linked directly by the bonds in question but also on the rest of the molecule. However, these effects are usually small, and it would be very difficult to take them into account in a general way. The classical procedure in deriving bond energies, i.e., to evaluate average values for all like bonds, has therefore been used by Pauling.[115c] Some of his values are given in Table XXXV.

<div align="center">

TABLE XXXV

ENERGY VALUES FOR COVALENT BONDS

</div>

Bond	Bond Energy kcal./mole	Bond	Bond Energy kcal./mole
H—H	103.4	C—Cl	66.5
C—C	58.6	C—Br	54.0
N—N	23.6	C—I	45.5
O—O	34.9	C=C	100
S—S	63.8	C=O	
		Formaldehyde	142
C—H	87.3	Other	
		aldehydes	149
N—H	83.7		
		Ketones	152
O—H	110.2		
S—H	87.5	C=N	94
C—N	48.6	C=S	103
C—O	70.0	C≡C	123
C—S	54.5	C≡N	150

The assumption of additivity of bond energies has been found by empirical test to be justified in most cases, and values for heats of formation obtained by adding the bond energies agree with the experimental figures within a few kilocalories. Superimposed on this additivity are the effects of the remainder of the molecules, which have been extensively studied in recent years.

Pauling and Sherman[144] have empirically determined the stabilizing energies contributed by resonance to a number of organic compounds. They compared the observed energies of formation with those empirically calculated on the assumption of additivity of bond energies; for those molecules in which resonance was expected from other considerations, the observed energies of formation were greater than the calculated. The difference in the two values for a given compound was considered to be the resonance energy.

The resonance energies for a few compounds, taken from Pauling,[115c] are given in Table XXXVI.

[144] Pauling and Sherman, *J. Chem. Phys.*, **1**, 606 (1933).

TABLE XXXVI

RESONANCE ENERGIES OF ORGANIC COMPOUNDS

Substance	Resonance Energy kcal./mole	Substance	Resonance Energy kcal./mole
Benzene	39	Thiophene	31
Naphthalene	75	Acids	28
Anthracene	105	Esters	24
Phenanthrene	110	Amides	21
Biphenyl	8*	Urea	37
Stilbene	15*	Phenol	7*
Pyridine	43	Phenyl cyanide	5*
Pyrrole	31	Carbon monoxide	58
Furan	23	Carbon dioxide	33

* In excess of that for the benzene rings.

Bent and co-workers have made a study of the equilibria between sodium and various triarylmethyl radicals and have calculated the strength of the C—C bond in hexaarylethanes. Bent and Ebers [145] conclude that, in dixanthyls, the C—C bond is weakened by about 16 kcal.; this they attribute to a combination of steric and resonance effects. From studies of heats of reactions, Bent, Cuthbertson, et al.,[146] conclude that the C—C bond in hexaphenylethane is about 30 kcal. weaker than a normal C—C bond, and that this is due about equally to the weakening of the C—C bond in the ethane and to the stabilizing effect of the resonance energy of triphenylmethyl.

A series of accurate measurements of the heats of hydrogenation of some unsaturated organic compounds has been made by Kistiakowsky, Vaughan, et al. The results of the investigations have been summarized by Conant and Kistiakowsky.[147]

The heat of hydrogenation of ethylene is 32.8 kcal./mole. Substitution of the hydrogen atoms by alkyl groups was found to lower the heat of hydrogenation progressively until for tetramethylethylene $\Delta H = 26.9$ kcal. The effect of substitution is not additive, each succeeding substituent having less effect than the preceding, and is independent of the chain length of the normal alkyl radical substituted. cis-Butene-2 has a heat of hydrogenation of 28.6 kcal., while that for trans-butene-2 is 27.6 kcal.; the value for cyclohexane is the same as that for cis-butene-2.

The heats of hydrogenation of the first bonds of a number of compounds containing multiple double bonds have been determined and the
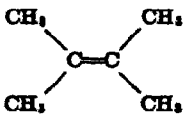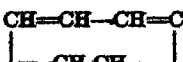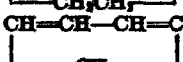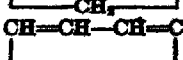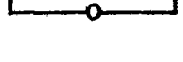
[145] Bent and Ebers, J. Am. Chem. Soc., 57, 1242 (1935).
[146] Bent, Cuthbertson, et al., ibid., 58, 165, 170 (1936).
[147] Conant and Kistiakowsky, Chem. Rev., 20, 181 (1937).

data analyzed. The heat effect is greatest for 1,4-addition, there being an exaltation of 2 to 3 kcal. due to conjugation; substitution on a terminal carbon atom of butadiene-1,3 has about the same effect as substitution on ethylene. When addition is in the 1,2-position, the effect of the second double bond in butadiene-1,3 is similar to that of an alkyl group on ethylene. When one mole of hydrogen is added to an unconjugated diene, ΔH is only slightly higher than that for the hydrogenation of propylene.

In Table XXXVII, taken from Conant and Kistiakowsky, are given the heats of reaction and free energy changes accompanying some hydro-

TABLE XXXVII

VALUES OF ΔF^0 FOR THE ADDITION OF ONE MOLE OF HYDROGEN AT 298° K. FOR SOME UNSATURATED HYDROCARBONS

(Gaseous state)

Compound	Adden- dum	Product	298° K.		
			ΔH	$-T\Delta S^0$	ΔF^0
$CH_2=CH_2$	H_2	C_2H_6	-32.6	8.2	-24.4
$CH_3CH=CH_2$	H_2	$CH_3CH_2CH_3$	-29.9	8.9	-21.0
$CH_3CH=CHCH_3$ (cis)	H_2	$CH_3CH_2CH_2CH_3$	-28.2	8.9	-19.4
(CH₃)₂C=C(CH₃)₂ [see structure]	H_2	$(CH_3)_2CHCH(CH_3)_2$	-26.4	8.9	-17.5
$C_6H_5CH=CH_2$	H_2	$C_6H_5CH_2CH_3$	-28.3	8.9	-19.5
$CH_2=CH—CH=CH_2$	H_2	$CH_3CH=CHCH_3$ (trans)	-29.2	8.9	-20.3
CH=CH—CH=CH (cyclo, CH₂CH₂)	H_2	CH=CH—CH₃ / CH₂CH₂CH₃	-26.5	8.8	-17.7
CH=CH—CH=CH (cyclo, CH₂)	H_2	CH=CH—CH₂—CH₂ / CH₂	-23.7	8.8	-14.8
CH=CH—CH=CH (cyclo, O)	H_2	CH₂—CH₂—CH=CH / O	-11.4	8.9	-2.5
C_6H_6	H_2	[cyclohexene structure with H₂ H₂]	5.8	7.8	13.6

genation reactions. The free energies of the reactions determine if the reaction as written will proceed; if ΔF is about -4 kcal., the reaction goes 99 per cent to completion at equilibrium; if ΔF is $+4$ kcal., only 1 per cent of the product is formed.

With the exception of furan and benzene, all the unsaturated compounds given in Table XXXVII hydrogenate completely. The first double bond of benzene does not hydrogenate completely under normal conditions of temperature and pressure, since the equilibrium is very much in favor of the reactants; under the same conditions, the addition of hydrogen to cyclohexadiene can proceed to completion. Cyclohexadiene cannot be obtained by the direct hydrogenation of benzene, since the conditions under which the first mole of hydrogen adds favor the complete addition of the second and third moles. Furan behaves like benzene.

It is possible to calculate the thermodynamic properties of simple compounds from spectroscopic data. Such calculations have been made for some organic compounds, and the results agree with those obtained by means of direct thermochemical measurements. Examples of such calculations for methane derivatives are given by Stevenson and Beach,[148] and for hydrocarbons by Pitzer [138, 149] and Aston.[129] From such derived data, equilibrium constants for reactions involving hydrocarbons, e.g., splitting or cracking reactions, can be computed; such computations are of value, inasmuch as the direct measurements of gaseous equilibria involving mixtures of hydrocarbons are extremely difficult. Examples of constants so calculated by Pitzer are given in Table XXXVIII.

TABLE XXXVIII

EQUILIBRIUM CONSTANTS FOR HYDROCARBON REACTIONS

Reaction	K_{298}	K_{600}	K_{1000}
$n\text{-}C_4H_{10} = iso\text{-}C_4H_{10}$	2.5	0.7	0.4
$C_2H_6 = C_2H_4 + H_2$	1.3×10^{-15}	1.7×10^{-4}	5
$C_3H_8 = CH_4 + C_2H_4$	7×10^{-8}	1.0	6×10^2
$n\text{-}C_nH_{2n+2} = C_2H_4 + n\text{-}C_{n-2}H_{2n-2}$ ($n > 7$)	3×10^{-10}	0.09	170
$(CH_3)_3CCH_2CH(CH_3)_2 = iso\text{-}C_4H_{10} + iso\text{-}C_4H_8$	9×10^{-8}	7	5×10^3
$n\text{-}C_7H_{16} = (CH_3)_2CHCH_2CH_2CH_2CH_3$	7	1.6	1.0
$n\text{-}C_7H_{16} = (CH_3)_3CCH_2CH_2CH_3$	29	0.7	0.2
$n\text{-}C_7H_{16} = (C_2H_5)_3CH$	1.1	0.4	0.3
$n\text{-}C_7H_{16} = (CH_3)_3CCH(CH_3)_2$	10	0.4	0.1

Calculations of thermodynamic quantities from spectroscopic data have recently afforded a measure of the potential energy hindering free rotation in single C—C bonds. The results for a number of compounds, as summarized by Aston, are given in Table XXXIX.

148 Stevenson and Beach, *J. Chem. Phys.*, **6**, 25, 108, 341 (1938).

149 Pitzer, *ibid.*, **5**, 469 (1937).

TABLE XXXIX

POTENTIALS HINDERING INTERNAL ROTATION IN CERTAIN COMPOUNDS AS
ASCERTAINED FROM LOW-TEMPERATURE THERMAL DATA

Compound	Potentials calories	Compound	Potentials calories
Methyl alcohol	6400	Ethyl alcohol	3000
Tetramethylmethane	4500	Isopropyl alcohol	3400
Ethane	3150		5000
Methylamine	3000	Acetone	1000
Propane	3300	Dimethylamine	3460
		Dimethylacetylene	0

Acknowledgment: Part of the material in this chapter was taken from the corresponding chapter of the first edition of this work. The authors are indebted to Professor Wallace R. Brode for permission to use this matter.

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CHAPTER 24

THE REDISTRIBUTION REACTION

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CONTENTS

INTRODUCTION

It is pointed out elsewhere (p. 1072) by Adkins that "there are few, if any, simple equilibria among organic reactions." This unquestioned fact epitomizes the fundamental complexity of the usual organic chemical processes, their irreversibility and confusion of side reactions: all, no doubt, a consequence of the fact that the bonds involved are as a rule largely covalent in type, and their dissociation tends to result in the formation of unstable, reactive radicals or molecular fragments.

Despite this general rule, it has recently been found that there does exist a very simple type of organic equilibrium, which occurs in several different classes of compounds at least, and which is amenable to experimental study. The process leading to this equilibrium has been termed the "redistribution reaction." Although only a limited amount of work has been done, the results are sufficient to point out unusual opportunities for investigation of the behavior of the covalent bond and the mechanism of organic reactions in the liquid phase.

METATHETICAL REACTIONS AND THEIR EQUILIBRIA

In the organic field, there are a relatively small number of examples of equilibrium reactions of the general type symbolized by:

$$AY + BZ \rightleftarrows AZ + BY \tag{1}$$

These metathetical reactions are commonplace in inorganic chemistry, where ionizing solvents bring about the dissociation of electrovalent salts into their ions. For the covalent bonds in organic compounds, this does not take place so readily and the presence of an inorganic reagent or catalyst is usually required to open up the bond. In the esterification and neutralization of organic acids, which are perhaps the most familiar examples of the above type of metathesis in organic chemistry, an ionic mechanism is also involved, but this is not necessarily so in other reactions.

The metathesis is possible only when the components, A, B, Y, and Z, if not atoms, are radicals which retain their structure and identity, after recombination. In this respect, the metathetical reactions differ basically from intramolecular rearrangements such as tautomerism, racemization, and cis-trans isomerization. Such rearrangements involve a more profound change in the molecular architecture since two separate chemical bonds in each molecule are simultaneously involved in the rearrangement.

Also, the A, B, Y, and Z components, if not ionized, at least retain sufficient polarity during the interchange to prevent the formation of the possible AA, AB, BB, YY, YZ, and ZZ compounds; that is, there is little or no side reaction.

As a result, under favorable reaction conditions (including if necessary the presence of a catalyst) an equilibrium can be reached, as indicated in equation 1. Often, the equilibrium is so far to one side as to be entirely unobserved in practice, but there remain a number of them which can be measured, and established by an approach from both sides. In most of these, the equilibrium position lies on one side or the other of that "mid-point" at which both the forward and the reverse reactions have the same unit velocity. Finally, in most of the few remaining instances where the equilibrium (at some particular temperature) is just at this mid-point, it is known that a change in the temperature will displace it in one direction or the other. As a general rule, the numerical value of the equilibrium constant and its temperature coefficient cannot be predicted, even from thermodynamic data, with any degree of assurance, if at all.

In most of the familiar metatheses, two different pairs of atoms are present in the bonds involved—in esterifications, for example, C—O and O—H bonds are formed and broken—and this facilitates the displacement of the equilibrium in one direction. On the other hand, when all the bonds broken are between the same atom pairs, the difference between the radicals A and B, and between Y and Z, is not manifested directly at the site of the bonds, and can influence the bonds only by causing an electron displacement in the bonding atom or by a steric effect. Under these circumstances there is less tendency for the equilibrium to be displaced far to one side or the other.

THE REDISTRIBUTION REACTION

In a series of papers by the present writers and their co-workers [1] it has been shown that there exist a number of liquid-phase equilibria of the above general type, which are free from disturbing side reactions, and which are distinctive in two respects: first, although the compounds involved may be wholly covalent in type, no ionizing solvent is required for reaction, although a catalyst is needed; second, the interchange takes place purely at random, and the composition of the product can

[1] (a) Calingaert, Beatty, Soroos, et al., J. Am. Chem. Soc., 61, 2748 (1939); (b) ibid., 61, 2755 (1939); (c) ibid., 61, 2758 (1939); (d) ibid., 61, 3300 (1939); (e) ibid., 62, 1099 (1940); (f) ibid., 62, 1104 (1940); (g) ibid., 62, 1107 (1940); (h) ibid., 62, 1542 (1940); (i) ibid., 62, 1545 (1940); (j) ibid., 63, 947 (1941).

be calculated in advance from the law of probability. This randomness implies that there are no directive effects of structure, or that the energy of each kind of bond involved is unaffected by the nature of the other bonds in the molecule. The opposing chemical forces are evenly balanced and the equilibrium composition does not change with temperature. This interchange process is the "redistribution reaction," and the product is termed a "random equilibrium mixture."

The study of the reaction to date comprises but a few examples among esters and aliphatic halides, and a larger number in the field of organometallic compounds. It appears probable, however, that many more examples await discovery. The following sections first describe the reaction and its conditions; next, discuss the composition of the products obtained; and finally, indicate the probable mechanism involved.

EXAMPLES OF THE REACTION

Esters and Aliphatic Halides. If an equimolecular mixture of ethyl acetate and methyl butyrate is heated, alone or with an inert, non-ionizing solvent, no reaction occurs; but, on the addition of a small amount of a catalyst such as aluminum ethylate, an interchange or redistribution of the acyl and alkoxy radicals takes place and leads to the equilibrium:[1a]

$$MeCOOEt + PrCOOMe \rightleftarrows MeCOOMe + PrCOOEt \qquad (2)$$

in which each of the four compounds is present in the same proportion, 25 mole per cent. Here the two acyl radicals, let us say, correspond to A and B in the type equation 1, and the two alkoxy radicals correspond to Y and Z; the bonds broken and formed are all carbon-oxygen. The forward and reverse reactions are evenly balanced and the two pairs of esters on either side have the same probability of existence in their mixture; that is, the radicals are distributed at random.

A similarly catalyzed redistribution takes place between furfuryl acetate and ethyl furoate, and also between dimethyl oxalate and dibutyl oxalate.[1i] The latter can also be written in conformity with the type equation 1, as follows:

$$MeO(CO \cdot COOMe) + BuO(CO \cdot COOBu) \rightleftarrows$$
$$MeO(CO \cdot COOBu) + BuO(CO \cdot COOMe) \quad (3)$$
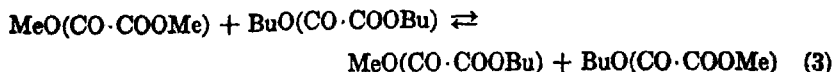
In this example the type AZ and BY compounds on the right-hand side of the equation happen to be identical, but this does not affect the randomness of the interchange. Hence, starting with equimolecular

quantities of the two symmetrical compounds, at equilibrium the mixture contains 25 mole per cent of each of these and 50 per cent of the methyl butyl oxalate.

In the halide field, an analogous redistribution is shown in the reaction of ethylene dihalides, catalyzed by aluminum chloride.

$$ClCH_2CH_2Cl + BrCH_2CH_2Br \rightleftarrows 2ClCH_2CH_2Br \qquad (4)$$
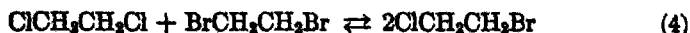
This reaction was observed by Dougherty,[2] who stated that it is reversible and that its product corresponds to "the laws of chance." The significance and generality of this statement were apparently not appreciated by subsequent readers. It is evident that two somewhat different bonds, carbon-chlorine and carbon-bromine, are concerned here, but in the two different organic radicals, $-CH_2CH_2Cl$ and $-CH_2CH_2Br$, the carbon atoms at the site of the ruptured bond are so nearly in the same chemical or electronic state as to be practically equivalent, with the result that the equilibrium is not displaced, but remains random. At least, the difference, if any, in energy between the $Cl-CH_2CH_2Cl$ and $Cl-CH_2CH_2Br$ bonds is equal to the difference between the $Br-CH_2CH_2Cl$ and $Br-CH_2CH_2Br$ bonds.

A redistribution also occurs between ethyl chloride and ethylene dibromide, or between ethyl bromide and ethylene dichloride.[14]

$$EtCl + BrCH_2CH_2Br \rightleftarrows EtBr + ClCH_2CH_2Br \qquad (5)$$
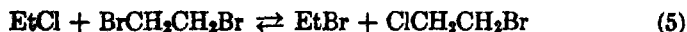
At the same time the ethylene halides interchange according to equation 4, so that the product contains each of the five possible different compounds.

Organometallic R_nM Compounds. Dimethylmercury and diethylmercury are well-defined and stable chemical entities; when pure, they can be mixed without undergoing any transformation, but in the presence of a suitable catalyst, such as methylmercury chloride, an interchange of methyl and ethyl radicals takes place[16] in accordance with the equation.

$$Me_2Hg + Et_2Hg \rightleftarrows 2MeEtHg \qquad (6)$$

This equation also can be written to conform with the general type given above, thus:

$$Me(HgMe) + Et(HgEt) \rightleftarrows Me(HgEt) + Et(HgMe) \qquad (7)$$

Hence, starting with equimolecular quantities of dimethyl- and diethylmercury, at equilibrium the mixture is found to contain 25 mole per cent of each of these and 50 per cent of methylethylmercury; starting with

[2] Dougherty, *ibid.*, **51**, 576 (1929).

pure methylethylmercury, the same equilibrium mixture is obtained. The latter reaction accounts for an observation made more than 80 years ago by Frankland,[3] who treated ethylmercury chloride with dimethylzinc and stated that: "It is probable that mercuric ethomethide was formed in the above reaction; but subsequent distillations gradually transformed it, more or less perfectly, into a mixture of mercuric methide and mercuric ethide."

Similarly, if an equimolecular mixture of tetramethyllead and tetraethyllead is treated with triethyllead chloride or aluminum chloride as the catalyst, a redistribution reaction takes place, as indicated by equation 8, to yield all the five possible R_4Pb compounds in the proportions given:[1a]

$$8Me_4Pb + 8Et_4Pb \rightarrow$$

$$1Me_4Pb + 4Me_3EtPb + 6Me_2Et_2Pb + 4MeEt_3Pb + 1Et_4Pb \quad (8)$$
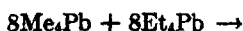
The result is, in effect, an equilibrium represented by the equation:

$$Me(PbR) + Et(PbR') \rightleftarrows Me(PbR') + Et(PbR) \quad (9)$$

where R and R' symbolize any two of the following four possible R_3 groups: Me_3, Me_2Et, $MeEt_2$, and Et_3. The numerical values of the concentrations of the five components in the product correspond to the law of probability, as will be explained below. In this example, the same product will be obtained by starting with dimethyldiethyllead alone, or with an equimolecular mixture of trimethylethyllead and methyltriethyllead, or with any other mixture containing altogether equal proportions of methyl and ethyl radicals.

Likewise, a mixture of tetramethyl-, tetraethyl-, and tetra-n-propyllead yields at equilibrium a product containing the fifteen possible R_4Pb compounds in proportions corresponding to a random distribution of the three radicals.[1a] Other examples of this reaction of RM compounds have been given where the reactants are two or more different radicals, including methyl, ethyl, n-propyl, isopropyl, isobutyl, $tert.$-butyl, phenyl, and p-tolyl, bound to one or more metals, including lead, tin, silicon, and mercury.[1a, a] Also, a high-temperature uncatalyzed interchange of radicals has been observed for a mixture of triphenyl- and tri-α-naphthylbismuth compounds.[4] As for zinc alkyls, although a mixture of dimethyl- and diethylzinc fails to show an appreciable amount of redistribution,[1e] the RR'Zn compounds are known to rearrange on heating to give a mixture of R_2Zn and R'_2Zn.[5]

[3] Frankland, *Ann.*, **111**, 59 (1859).

[4] Challenger and Ridgway, *J. Chem. Soc.*, **121**, 104 (1922).

[5] Krause and Fromm, *Ber.* **59**, 931 (1926).

Whenever compounds of two different metals react under similar conditions, as a rule they also react with one another, again yielding all the possible compounds of both metals. For example, when a mixture of tetramethyllead and diethylmercury reacts in the presence of an aluminum chloride catalyst, there is an interchange of the alkyl radicals between the two metals,[1h, i] which can be represented as follows:

$$Me(PbR_3) + Et(HgR) \rightleftharpoons Me(HgR) + Et(PbR_3) \tag{10}$$

At the same time there is a redistribution of the radicals between the R_2Hg compounds (equation 6) and between the R_4Pb compounds (equation 8) giving two independent random equilibrium mixtures containing all eight possible compounds. However, in the transfer of the radicals from one metal to the other (equation 10), the PbR_3 and HgR groups, corresponding to Y and Z in the type equation 1, are sufficiently unlike to cause the equilibrium to be displaced to the right, as indicated by the arrows. In other words, the difference in energy between the Me—HgR and Me—PbR_3 bonds is not equal to that between the Et—HgR and Et—PbR_3 bonds; instead the mercury shows a greater relative affinity than lead for methyl as compared with ethyl radicals.

The above interchange, therefore, is not strictly speaking a redistribution reaction, although it is in many respects similar, and may be regarded as a borderline case. On the basis of the law of mass action, the displacement of the equilibrium can be expressed by an equilibrium or "relative affinity" constant, K, whose value is given by $K = \dfrac{[Me-Hg]\,[Et-Pb]}{[Et-Hg]\,[Me-Pb]}$, where the brackets denote the proportions of the four different R—M bonds in the total product. This constant has to be experimentally determined on one occasion, after which its use enables the prediction of the equilibrium composition of any other system made up of the same two metals and radicals in other proportions. For the above example, tests made with several different mixtures have shown that $K = 4$, approximately.

In like manner, a mixture of tetraethyllead and tetramethyltin yields a product containing each of the ten possible R_4M compounds.[1a]

Organometallic Halides. A similar redistribution of alkyl radicals takes place in the R_3PbX compounds (X is a chlorine or bromine atom), and the reaction is spontaneous, since the reactants serve as their own catalyst. Thus, a mixture of trimethyl- and triethyllead chlorides, or the single compound, dimethylethyllead chloride, spontaneously reacts to yield the four possible R_3PbCl compounds in a random equilibrium mixture.[1j]

It follows that if, for example, tetramethyllead is treated with a triethyllead halide, there results an equilibrium mixture containing all the possible five R_4Pb and four R_3PbX compounds: [1]/

$$Me(PbR_3) + Et(PbR_2X) \rightleftarrows Et(PbR_3) + Me(PbR_2X) \qquad (11)$$

Also, the fact that trialkyllead chlorides act as catalysts here suggests the possibility of spontaneous interchange of the chlorine atom with the alkyl radicals from R_4Pb. The simplest possible reaction of this kind, where all the R radicals are identical, has been demonstrated by using radioactive lead (Pb*) as the tracer.[1d]

$$Et_4Pb^* + Et_3PbCl \rightleftarrows Et_3Pb^*Cl + Et_4Pb \qquad (12)$$
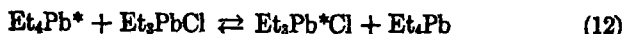
At the same time, there is very little formation of R_2PbX_2 compounds in these reactions [1e, f] indicating that the equilibrium

$$2R_3PbX \longleftarrow\rightleftharpoons R_4Pb + R_2PbX_2 \qquad (13)$$

is not a random distribution, but tends to go far to the left. At elevated temperatures, however, this equilibrium is displaced by the irreversible decomposition: [1e]

$$R_2PbX_2 \rightarrow R_2 + PbX_2 \qquad (14)$$

These reactions explain the apparent instability of the R_3PbX compounds. This instability and the spontaneous redistribution of the $R_2R'PbX$ types account for the observation of Grüttner and Krause [6] that, in the preparation of $R_2R'R''Pb$ compounds from $R_2R'PbX$ by the Grignard reaction, the halides should be used only when freshly prepared, as storage for any length of time leads to low yields and an impure product. The low yields are a result of the disappearance of part of the $R_2R'PbX$ (equations 13 and 14), while the impurities are other R_4Pb compounds derived from the various R_3PbX salts which were formed in the redistribution (equation 11). Similarly, in the synthesis of certain $R_3R'Pb$ compounds from the Grignard reaction on R_3PbX, some R_4Pb and $R_2R'_2Pb$ compounds are formed.[7]

Other Equilibria. In addition to the evenly balanced or random equilibria noted above, there are of course a number of reactions of somewhat similar types in which the equilibrium is usually displaced

[6] Grüttner and Krause, *Ber.*, 50, 202 (1917).
[7] Calingaert and Soroos, *J. Org. Chem.*, 2, 535 (1938).

more or less to one side. By definition, these do not fall within the scope of this chapter. The following are examples:

$$R_2M + MX_2 \rightleftarrows 2RMX \quad (M = Mg, Zn, or Hg)\,[8]$$

$$RK + R'H \rightleftarrows RH + R'K \quad (R,R' = aryl)\,[9]$$

$$MeCOOR + R'OH \rightleftarrows MeCOOR' + ROH\,[10]$$
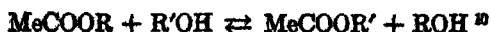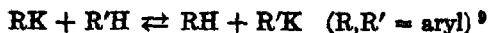
It may be noted that in the last of these, the alcoholysis of esters, there are a number of examples having equilibrium constants very close to unity, which may therefore be considered to be true redistribution reactions.

Reaction Conditions. The reactions have been carried out in the liquid phase at temperatures from 20° to 180° for five hours or less. To obtain the desired temperature, and at the same time to provide stirring and exclude air, it is convenient to add 50 ml. of an inert solvent such as hexane or decalin to 0.3 mole of the compounds, and to reflux the mixture. A change in temperature has a marked effect on the reaction velocity, as might be expected, whereas the presence of an inert solvent does not; neither variable has any observable influence on the composition of the completely reacted product.

In the absence of a catalyst no reaction whatever is detected, except at a high temperature for RM compounds where the R's are aryl groups. In general, the catalysts for organometallic compounds are organometallic halides, RMX, and contain, in a polar state, the radicals or atoms to be interchanged, or else (like aluminum chloride) the catalysts react with the substrates present to yield such RMX compounds. For the esters, the only catalyst which has been used is aluminum ethoxide, and for the aliphatic halides, aluminum chloride. Doubtless numerous other compounds would also be effective.

For the organometallic redistributions as well, aluminum chloride has generally been used, but a number of other agents have been tested in the Me$_4$Pb—Et$_4$Pb reaction, and a catalytic effect is also shown, for example, by the chlorides of zinc, mercury, zirconium, tin, phosphorus, arsenic, bismuth, iron, and platinum, and by zinc and boron fluorides. The activity of these different catalysts varies greatly.[1a] As little as 0.5 per cent dimethylaluminum chloride effects complete redistribution of R$_4$Pb in one-half hour at 25°, whereas the trialkyllead halides are

[8] Gilman, This Treatise, pp. 497, 547, 551.
[9] Conant and Wheland, J. Am. Chem. Soc., **54**, 1212 (1932).
[10] Fehlandt and Adkins, ibid., **57**, 193 (1935); Hatch and Adkins, ibid., **59**, 1694 (1937).

relatively weak catalysts, from 5 to 10 per cent being required to bring about complete reaction in five hours at 80°.

For the redistribution between tetramethyllead and tetraethyllead, the reaction is strictly quantitative,[1c] apart from a small amount of irreversible side reaction with the catalyst. Using one mole per cent of aluminum chloride as the catalyst, it is found that 1.5 per cent of the R_4Pb reacts [11] to yield R_3PbCl and, in small part, $PbCl_2$; if as much as 5 per cent dimethylaluminum chloride is used as the catalyst, only 0.2 per cent of the lead is converted to chlorides. In neither case is there any decomposition, evolution of gas, or precipitation of metallic lead. With less stable compounds such as the *tert.*-butyl R_4Pb, some decomposition may occur.

COMPOSITION OF RANDOM EQUILIBRIUM MIXTURES

Use of Law of Probability. In the type equation 1, if the distribution is purely random this result can be expressed by a conventional equilibrium constant equal to unity. Likewise the first of the examples given (equation 2) where four different esters are involved has a constant of 1. If equations 4 and 6, for the ethylene dihalide and R_2Hg reactions, were written in the form of equations 3 or 7, where an artificial distinction is made between the two identical molecules on the right-hand side, then their constant would likewise be equal to 1; in their present form, however, the constant is equal to 4. In reactions involving groups of higher valence, this conventional method of expressing the composition of the reaction product is not too satisfactory since a single equilibrium constant is insufficient. For example, in equation 9 for R_4Pb compounds the equilibrium has to be broken up into three different parts with separate constants for each. Equation 5 for ethyl halides with ethylene dihalides presents a similar difficulty.

On the other hand, for the present redistribution reactions the single criterion of randomness of interchange permits a simple and direct approach to the problem of representing and determining the equilibrium composition. All that is required is to express the randomness in terms of the law of probability, and to substitute in the resulting expression for the composition the appropriate numerical values for any given example. The expressions obtained [1a] reduce to rather simple forms for systems containing few constituents. For example, in the reaction given in equation 9, let $[Me] = r$, where $[Me]$ is the mole fraction of methyl radicals in the total of methyl plus ethyl; then the composition

[11] Gilman and Apperson, *J. Org. Chem.*, **4**, 162 (1939).

of the random equilibrium mixture which results from the reaction of *any* initial mixture or compound is given, in mole fractions, by:

$$[Me_4Pb] = r^4$$

$$[Me_3EtPb] = 4r^3(1 - r)$$

$$[Me_2Et_2Pb] = 6r^2(1 - r)^2$$

$$[MeEt_3Pb] = 4r(1 - r)^3$$

$$[Et_4Pb] = (1 - r)^4$$

For example, if $r = 0.5$, corresponding to equal proportions of methyl and ethyl radicals, the composition will be that given in equation 8. In general, from a knowledge of the composition of the original mixture, or from the over-all analysis of the product, the value of r is determined, and the expected concentrations in the final mixture can be calculated.

Experimental Tests of Me₄Pb-Et₄Pb Systems. For the equilibrium which has been most carefully studied, that of the methyl and ethyl R₄Pb compounds, the experimental agreement with the probability law is within the experimental error of the analyses. For this system, the analytical procedure (a vacuum fractional distillation) was tested with two synthetic mixtures of the five compounds and showed an average error of 0.6 per cent, whereas the analyses of eight different equilibrium mixtures, ranging from 20 per cent to 74 per cent methyl radicals, gave an average deviation of 0.5 per cent from the calculated composition.[1b] As an illustration, Figs. 1 and 2 show the experimental distillation curves [1a] of three different random equilibrium mixtures, in comparison with the values calculated from the law of probability.

The equilibrium might be expected to be more exactly random for isolated molecules in the gas phase than for the liquid phase. However, Allen [12] has shown that the vapor-pressure correction relating the gas and liquid phase equilibria is negligibly small for a series of homologous compounds such as the present R₄Pb system.

Predicted Results. Equilibrium constants for unmeasured reactions can sometimes be determined by calculation from known equilibria for an appropriate series of reactions connecting the various components of the given system. The present redistribution reactions provide values which may prove to be useful for this purpose. Conversely, it may become possible to predict other redistribution reactions. Thus, for equation 2 involving the methyl and ethyl acetates and butyrates, as pointed out by Stiehler and Gresham,[13] the combination of the four

[12] A. O. Allen, private communication.
[13] Stiehler and Gresham, *J. Am. Chem. Soc.*, **62**, 2244 (1940).

appropriate esterification constants of the corresponding acids would give an equilibrium constant for the redistribution, and only if it were equal to unity would the redistribution be truly random. Practically, it is questionable whether, even in this favorable example, the constants
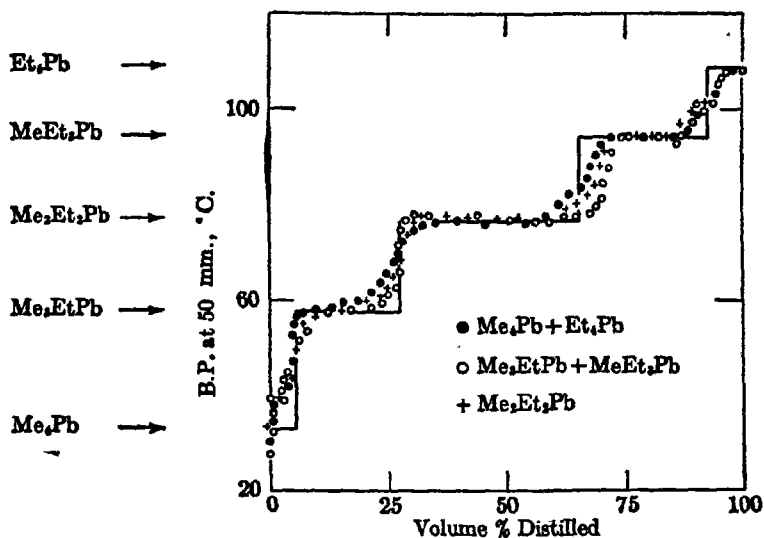
Fig. 1.—Distillation of equilibrium mixtures where [Me] = 0.5.
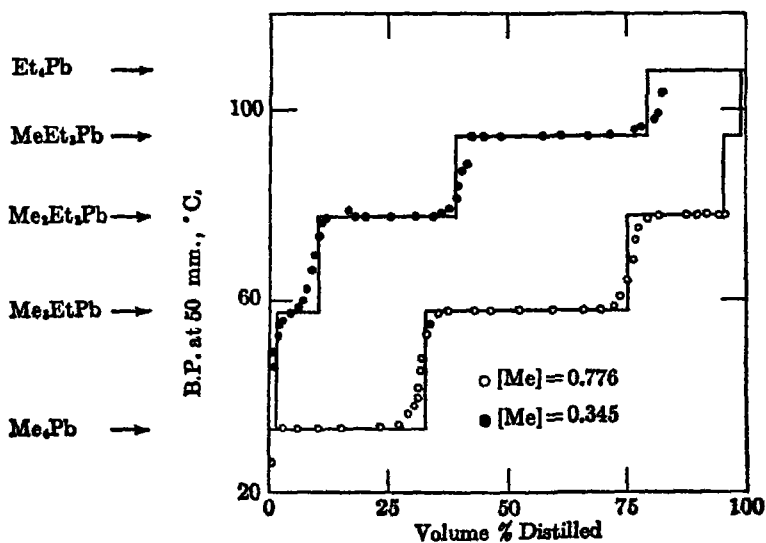
Fig. 2.—Distillation of equilibrium mixtures.

are known accurately enough to permit a calculation which is as reliable as the result furnished by the direct experiment and analysis of the product.

It is also possible to attempt a prediction of the equilibria based on molecular data. For such reactions as the interchange of the methyl and ethyl R_4Pb compounds where the bonds broken and formed are nearly identical, it may perhaps be legitimate to assume in advance that ΔH is practically zero; * if so, the equilibrium composition is determined solely by the change in entropy, ΔS°. On this basis, for examples of the present R_4M type, Stearn [14] has calculated from statistical mechanics that the steric effects involved in the interchange can make no more than an experimentally inappreciable contribution to ΔS°, so that the value of ΔS° is essentially merely that of the entropy of random distribution of the radicals.

MECHANISM AND KINETICS

A mechanism involving ions or free atoms or free radicals is unlikely, although it must be admitted that but little is known regarding the chemical properties of these reactive intermediates in the liquid phase or of reaction mechanisms involving them.[15] The heavier radicals, such as phenyl or R_3Pb, may be sufficiently stable under the conditions of reaction to permit direct interchange, and this would account for the non-catalyzed redistribution of tetraphenyl- and tetra-p-tolyllead, or of triphenyl- and tri-α-naphthylbismuth. However, it is to be expected that the lighter methyl and ethyl radicals, if liberated, would undergo secondary reactions, such as combination and disproportionation, which are completely absent in the redistribution reaction. In this connection, it is of interest to note that when a mixture of hexamethyldilead and hexaethyldilead, without added catalyst, is decomposed thermally in quantitative accord with the equation $2R_6Pb_2 \rightarrow 3R_4Pb + Pb$, the product contains all five possible R_4Pb compounds.[16] Also, it is significant that in the redistributions of R_4Pb mixtures containing on the one hand methyl and n-propyl radicals, and on the other, methyl and isopropyl radicals, there is no isomerization of the propyl groups in either reaction.[16]

A clue to the mechanism lies in the fact that the catalyst is always a compound of a type which is known to be capable of reversible ex-

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* See, however, Johnson, This Treatise, p. 1893.
[14] Stearn, *J. Am. Chem. Soc.*, **62**, 1630 (1940).
[15] Brown, Kharasch, and Chao, *ibid.*, **62**, 3435 (1940).
[16] Calingaert, Soroos, and Shapiro, *ibid.*, **64**, 462 (1942).

change of radicals or atoms with the materials undergoing redistribution, or which will form such a compound in the reaction mixture. For example, the following reactions have been established, the redistribution catalyst being underlined in each case:

$$\underline{AlCl_3} + R_4Pb \rightleftarrows RAlCl_2 + R_3PbCl,$$

$$\underline{RAlCl_2} + R_4Pb \rightleftarrows R_2AlCl + R_3PbCl \ [11]$$

$$\underline{AlCl_3} + 3RBr \rightleftarrows AlBr_3 + 3RCl \ [17]$$

$$\underline{R_3PbCl} + R_4Pb^* \rightleftarrows R_3Pb^*Cl + R_4Pb \quad \text{(equation 12)}$$

$$\underline{Al(OR)_3} + 3MeCOOR' \rightleftarrows Al(OR')_3 + 3MeCOOR \ [18]$$
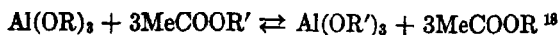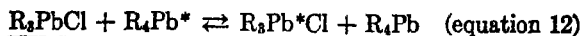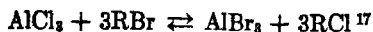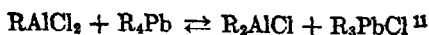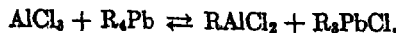
This suggests that the mechanism of the redistribution reaction may be the reversible formation of a loose complex between a molecule of the catalyst and one or more molecules of the compounds to be redistributed. When the complex breaks up, each molecule is re-formed with its proper complement of groups taken at random from the common supply in the complex, and the final result of the repetition of this process is an equilibrium corresponding to complete random redistribution. The information obtained so far on the redistribution reaction is in accord with such a mechanism, but does not yet include data which confirm its correctness. Further insight can be obtained from properly designed kinetic studies, where the reaction is stopped before reaching equilibrium and the products are analyzed.

For example, in the redistribution of R_4Pb compounds, it is possible that the alkyl radicals are interchanged either one at a time or in pairs. In either event, the final equilibrium will be the same, but during the reaction the change of composition as a function of time will be noticeably different.

In unpublished work in the writers' laboratory, the differential equations have been set up and integrated to give the composition, as a function of time, resulting from the interchange of radicals one at a time, for any initial concentration of one or more of the five R_4M compounds where there is one M group with two kinds of R's. To take a simple example, if the initial composition is an equimolecular mixture of tetramethyl- and tetraethyllead, the equations reduce to:

$$[Me_4Pb] = [Et_4Pb] = \tfrac{1}{16}(1 + 6e^{-t/2} + e^{-t})$$

$$[Me_3EtPb] = [MeEt_3Pb] = \tfrac{1}{4}(1 - e^{-t})$$

$$[Me_2Et_2Pb] = \tfrac{3}{8}(1 - 2e^{-t/2} + e^{-t})$$

[17] Pouret, Bull. soc. chim., [3] 25, 295 (1901).
[18] Baker, J. Am. Chem. Soc., 60, 2673 (1938).

Here, the unit of time, t, is numerically indeterminate, being simply the time required for an average of one random interchange of a radical for each single R_4M molecule present. When these three equations are plotted, as in Fig. 3, it is seen how the composition progresses toward equilibrium, and it is evident that the determination of the concentration of any one of the five components at some particular time defines
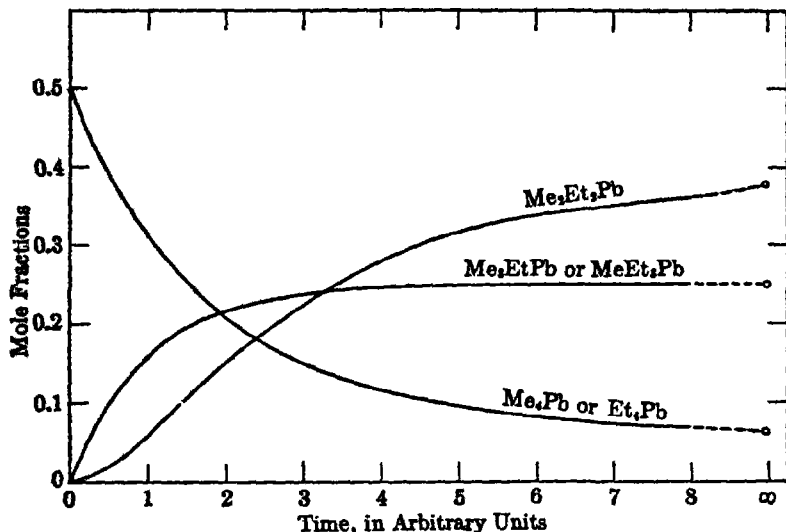
FIG. 3.—Approach to equilibrium.

the composition of the whole mixture, provided that the reaction follows the postulated mechanism. In other words, the reaction serves as its own clock or time-measuring device. This means that the reaction velocity not only does not have to be measured in the usual way but also does not even have to be held constant. Thus, for example, a change of activity of the catalyst during the reaction is of no concern. Actually, in a number of such experiments which were made by interrupting the reactions at different times, the results of the analyses of the reaction mixtures failed to show reasonable agreement with these equations. Hence it is believed that the reaction mechanism is not confined to the simple interchange of one radical at a time.

CHAPTER 25

MODERN ELECTRONIC CONCEPTS OF VALENCE

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CONTENTS

INTRODUCTION

The evolution of valence theories in organic chemistry has been accompanied by the introduction of characteristic symbols that have been applied with considerable success in the interpretation of structural phenomena and of organic chemical reactions. The notion of integral valence bonds and the genesis of structural formulas, together with the recognition of a localized distribution of valence forces in space, has been sufficient to account in large measure for diverse types of isomerism and in general for the phenomena associated with organic molecules in an inactive or resting state. Efforts to account for dynamic effects in these systems have led to a resolution of integral valence bonds into more or less nebulous components through the assumption of partial valences,

primary and secondary valences, and residual fractional polarities. The contribution of these postulates to the growth and general progress of organic chemistry cannot be denied, but efforts to attach a precise physical significance to a valence unit or its hypothetical components had met with insuperable obstacles.

The formulation by Lewis [1] of a more explicit electronic concept of valence and molecular structure has laid the foundation for advances in the direction of expressing chemical affinities in terms of more clearly defined atomic and molecular models. The older theories of organic chemistry and earlier electronic formulations of organic reactions [2] have taken on more definite form, and a large number of chemical phenomena are seen from a new point of view.

The introduction of the electronic theory and its application to problems of molecular constitution were advanced by the contributions of Langmuir, [3] and in the period since 1920 a steady development and elaboration of the fundamental principles of the theory have taken place. Progress in the field of inorganic chemistry has been more rapid than in the organic domain, and many of the important generalizations and correlations have come from studies of the simpler inorganic molecules.

It is evident, however, that the electronic theory must be applicable to chemistry as a whole. A strong impetus to its general acceptance and development was given by Sidgwick, [4,5] who showed that the underlying principles of the theory and the contributions of modern developments in atomic physics may be used with remarkable success in interpreting the varied chemical behavior of covalent molecules and ionized salts and in elucidating the chemical relations of the elements in the periodic table.

The general application of modern electronic concepts to organic chemical reactions is due largely to Robinson [6] and Ingold, [7] and their collaborators. This subject has been developed extensively during the

[1] Lewis, *J. Am. Chem. Soc.*, **38**, 762 (1916); "Valence and the Structure of Atoms and Molecules," Chemical Catalog Co., New York (1923); see, also, *Chem. Res.*, **1**, 231 (1925); *J. Chem. Phys.*, **1**, 17 (1933).

[2] Fry, "The Electronic Conception of Valence and the Constitution of Benzene," Longmans, Green and Co., New York (1921). This monograph contains a review of the early applications of electronic concepts to chemical reactions.

[3] Langmuir, *J. Am. Chem. Soc.*, **41**, 868, 1543 (1919); **42**, 274 (1920); *Ind. Eng. Chem.*, **12**, 386 (1920).

[4] Sidgwick, "The Electronic Theory of Valency," Oxford University Press, Oxford (1929).

[5] Sidgwick, "The Covalent Link in Chemistry," Cornell University Press, Ithaca, N. Y. (1933); see, also, *Ann. Repts. Chem. Soc. (London)*, **30**, 110 (1933); **31**, 37 (1934).

[6] Robinson, "Outline of an Electrochemical (Electronic) Theory of the Course of Organic Reactions," Institute of Chemistry of Great Britain and Ireland, London (1932).

[7] Ingold, *J. Chem. Soc.*, 1120 (1933); *Chem. Rev.*, **15**, 225 (1934).

past decade but is not yet beyond the state of a roughly qualitative solution of the varied and complex problems of organic chemistry. A good deal of progress has been made by way of adaptation and refinement of the earlier views, and further advances are forthcoming especially in the quantitative aspects of the subject.

From the mathematical and physical side, problems of atomic and molecular structure have been approached through the development and elaboration of the theory of quantum mechanics.[2] This has led to the notion of quantum-mechanical resonance (p. 1943)—resonance of a molecule among several valence bond structures—which has been applied extensively to organic molecules by Pauling.[3]

The Electronic Configuration of Atoms. The atom is pictured as made up of a dense nucleus and a complement of electrons. The nucleus bears a net positive charge equal to the atomic number of the element and is surrounded by a quantity of electrons sufficient to neutralize the nuclear charge. In the classical theory of Bohr the electrons are considered to revolve about the nucleus in concentric orbits or shells (quantum groups).[4] On the basis of physical investigations Bohr grouped atoms into four classes: (I) those in which all the shells contain their full complement of electrons (the inert gases); (II) those in which all but the highest quantum group (outermost shell) is complete; (III) those in which the two outermost electronic groups are incomplete (the transitional elements); (IV) those in which the three outermost electronic groups are incomplete (the rare-earth elements).

The chemical properties of an atom are determined essentially by the electrons in the outermost shell, designated as valence electrons, which for a free atom never exceed eight in number. The underlying shells and the nucleus constitute the *kernel,* or effective nucleus, of the atom, which remains unaltered in all ordinary chemical changes. In hydrogen the kernel is a proton and has a unit positive charge; in the elements of the first short period, from lithium to fluorine, the kernel includes two planetary electrons (the helium pair) and consequently has a net positive charge, called the effective nuclear charge, of two less than the atomic number. In the elements of the second short period, from sodium to chlorine, the kernel includes ten planetary electrons in two shells (the helium pair plus an octet), and the effective nuclear charge is ten less than the atomic number. The atoms most frequently

[2] Pauling and Wilson, "Introduction to Quantum Mechanics, with Applications to Chemistry," McGraw-Hill Book Co., New York (1935).

[3] Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y. (1939).

[4] For the quantum-mechanical treatment of the motion of electrons by means of orbital wave functions, see Chapter 26, p. 1952 ff.

encountered in organic compounds are included in these two periods, in which the number of valence electrons (and the effective nuclear charge) increases regularly from one to seven and corresponds to the group of the element in the periodic table.

TABLE I

OUTERMOST SHELLS OF TYPICAL ELEMENTS

Number of electrons	1	2	3	4	5	6	7	8
Hydrogen period	H	He						
First short period	Li	Be	B	C	N	O	F	Ne
Second short period	Na	Mg	Al	Si	P	S	Cl	A
First long period	K	Ca	Ga	Ge	As	Se	Br	Kr
Second long period	Rb	Sr	In	Sn	Sb	Te	I	Xe
Third long period	Cs	Ba	Tl	Pb	Bi	Po		

TABLE II

ELECTRONIC CONFIGURATIONS OF THE INERT GASES

Atom and Atomic Number	Electrons in Quantum Groups					
	1st	2nd	3rd	4th	5th	6th
Helium (2)	2					
Neon (10)	2 + 8					
Argon (18)	2 + 8 + 8					
Krypton (36)	2 + 8 + 18 + 8					
Xenon (54)	2 + 8 + 18 + 18 + 8					
Radon (86)	2 + 8 + 18 + 32 + 18 + 8					

The extraordinary inactivity of the inert gases leads naturally to the fundamental postulate that the electronic configurations of these atoms represent the highest degree of symmetry and stability. Helium is characterized by a group of two electrons in the outermost shell, and the inert gases of higher atomic number by an outer shell of eight electrons. The systems of other atoms have a strong tendency either to give up electrons or take on additional electrons, in such a way as to approach the stable configuration of an inert gas. The tendency of electrons to form pairs (rule of two) and groups of eight (octets) is a basic principle of the theory of chemical combination. In general, the atoms with an effective nuclear charge of one or two tend to give up electrons (electropositive atoms), and those with a charge greater than two tend to acquire electrons and build up octets (electronegative atoms).

Ionic and Covalent Bonds. The union of atoms in a molecule may be effected through two different kinds of interatomic forces, electrovalence or covalence. An electrovalent or ionic bond is formed by the

complete transfer of an electron from one atom to another, and the binding force is due essentially to electrostatic attraction between the

TABLE III

BOHR'S CLASSIFICATION OF THE ELEMENTS

oppositely charged ions. The cation and anion can each be assigned a definite electronic structure essentially independent of the presence of the other, and this characteristic structure is retained as the ions ap-

proach one another to form a stable crystal. Thus, a crystal of sodium chloride is not made up of discrete molecules of NaCl but is an aggregate of sodium cations and chloride anions, in equal numbers, arranged so that each ion is equidistant from *six* neighboring ions of opposite charge.

In a covalent bond the atoms are held together by a pair of electrons which is shared by the two atoms and is considered to be effective in completing a stable electronic configuration for the valence shell of each atom. This type of bonding gives rise to discrete molecules having relatively weak external electrical fields. The forces of attraction between the distinct molecules (van der Waals' forces) are very weak in comparison with the Coulomb attraction of ions, and the distances between the atoms A and B belonging to different molecules are considerably greater than the interatomic distance within the discrete molecules A-B. Moreover, the binding force arising from a shared electron pair is localized and exerted in a definite direction about the atom, giving rise under appropriate conditions to stereochemical phenomena, whereas the electrostatic attraction of a free ion has no definite direction in space and extends to all ions of opposite sign in its neighborhood.

Sugden [10] has developed a theory based upon the hypothesis that atoms may be held together in stable combinations by the formation of a covalent bond in which only one electron is shared by the atomic nuclei, and he has used this assumption to formulate the electronic configurations of a number of inorganic molecules (SF_6, PF_5, etc.). There is evidence that hydrogen may form a one-electron covalent bond in the unstable hydrogen molecule-ion $[H_2]^+$, and in the boron hydrides, but it appears on physical grounds that covalent bonds of only one electron or of three shared electrons may be expected to occur only exceptionally (NO, NO_2, O_2, ClO_2, etc.) [11] There is certainly no justification for an assumption that covalent bonds of one or three electrons are present in stable organic molecules.

Coördinate Bonds. Covalent bonds may be considered to arise in two ways: each atom may contribute one electron of the binding pair (normal covalent link), or one of the two atoms may furnish both electrons (coördinate link). A bond of the second type is sometimes called a semi-ionic (semi-polar) or dative double bond. The coördination process involves the union of a *donor* atom possessing an unshared pair of electrons in its valence shell and an *acceptor* atom which is capable of holding two additional electrons. Typical donor atoms include 3-co-

[10] Sugden, "The Parachor and Valency," Routledge and Sons, London (1930); see, also, Chapter 23, p. 1744.

[11] Pauling, *J. Am. Chem. Soc.*, **53**, 3225 (1931); see, also, Chapter 26, p. 1960.

valent nitrogen in ammonia and amines, 2-covalent oxygen and sulfur, and 1-covalent iodine; examples of acceptor atoms include hydrogen cations (protons), 2-covalent magnesium and zinc in their alkyl derivatives, and 3-covalent boron in its alkyl derivatives and halides.

The union of triethylamine or diethyl ether with boron trifluoride serves to illustrate the formation of a coördinate link. Owing to the fact that nitrogen has five valence electrons it can complete its octet by forming three normal covalent bonds; the resulting 3-covalent nitrogen atom possesses two unshared valence electrons and can act as a donor. Boron has three valence electrons and can acquire but three more by the formation of normal covalent bonds, achieving a total of only six valence electrons. By virtue of the general tendency of a valence sextet to pass into the stable electronic configuration of an octet, 3-covalent boron can acquire two additional electrons and can act as an acceptor. In forming the compound R_3N-BF_3, the octet of boron is completed by the previously unshared electron pair of the nitrogen.

$$
\begin{array}{ccc}
\text{Et} & \text{F} & \text{Et F} \\
\ddot{\text{Et}}:\ddot{\text{N}}: & + \ddot{\text{B}}:\text{F} & \rightarrow \text{Et}:\ddot{\text{N}}:\ddot{\text{B}}:\text{F} \quad \text{(white crystalline solid)} \\
\text{Et} & \text{F} & \text{Et F} \quad \text{m p. 29.5}^\circ
\end{array}
$$

$$
\begin{array}{ccc}
& \text{F} & \text{F} \\
\text{Et}:\ddot{\text{O}}: & + \ddot{\text{B}}:\text{F} & \rightarrow \text{Et}:\ddot{\text{O}}:\ddot{\text{B}}:\text{F} \quad \text{(colorless liquid)} \\
\text{Et} & \text{F} & \text{Et F} \quad \text{b. p. 126}^\circ
\end{array}
$$
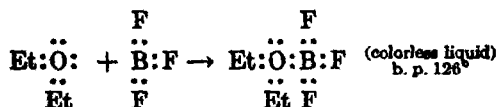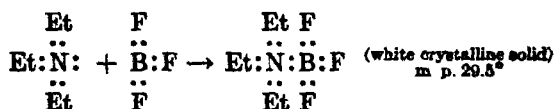
As a result of the coördination process, the acceptor atom obtains a share in two more electrons and its residual positive charge is decreased, while that of the donor atom is increased a corresponding amount. It is convenient to indicate the state of electrification in the resulting coördinate bond by assigning *formal charges* to the atoms, on the assumption (as a first approximation) that the electrons of the covalent bonds are divided equally between the bonded atoms. In the compound R_3N-BF_3 this calculation (see page 1833) gives a formal charge of $+1$ for the nitrogen atom and -1 for the boron atom. A coördinate link may thus be regarded as a double bond made up of one ionic bond of unit strength and one covalent bond, or as an intramolecular ion-pair (zwitterion).[12]

In general the formal charges do not represent the actual distribution of electrical charges among the atoms in a molecule or polyatomic ion, since the formal charge will be transferred in part to adjacent atoms

[12] N. V. Sidgwick, Chem. Rev., **17**, 1 (1935).

in the system. The actual extent of sharing of the electron pair in either coördinate or normal covalent bonds varies over a wide range in different links, and the distinction between them vanishes once the bond is established. In the example of coördination cited above it is sufficient to recognize that the nitrogen atom after coördination is in a condition similar to that in an ammonium ion, and the boron in a state similar to that in a fluoborate ion.

The formation of a coördinate link is denoted conveniently by means of an arrow drawn from the donor to the acceptor, $Et_3N \rightarrow BF_3$, showing the source of the electron pair and the orientation of the resulting dipole ($\overset{\delta+}{\underline{\hspace{1cm}}} \overset{\delta-}{\longrightarrow}$). This symbol refers merely to the manner of establishing the bond and serves mainly to aid in recognizing a condition in which the covalence of an atom exceeds the number of electrons it can contribute in the formation of the links. The symbol is useful also to indicate a situation in which the covalence of an atom exceeds the number of electrons it can contribute in the formation of the links or the number required to complete a stable group of eight (two, for hydrogen), and the higher covalent state is not accompanied by the appearance of definite ionic charges.

If one of the participants in the coördination is a univalent ion the integral charge is dissipated and the use of a distinctive symbol in the product is superfluous. When a molecule of ammonia undergoes coördination with a proton, the unit positive charge of the proton is distributed throughout the resulting ammonium ion and the new covalence becomes identical with a normal covalence. Likewise, the coördination of boron trifluoride with a fluoride ion produces a new anion in which the unit negative charge permeates the entire system and all the fluorine atoms in the fluoborate ion are held by identical covalences. The normal state of ammonium or fluoborate ions is not represented as a coördination complex (I, III) but as a system held together by ordinary covalent bonds (II, IV).

$$[\text{H}] \leftarrow\!\!\mid\!\text{NH}_3 \quad becomes \quad \left[\begin{array}{c} \text{H} \\ | \\ \text{H}-\text{N}-\text{H} \\ | \\ \text{H} \end{array}\right]^+ \qquad [\ddot{\text{F}}\dot{\cdot}]^- \!\!\rightarrow BF_3 \quad becomes \quad \left[\begin{array}{c} \text{F} \\ | \\ \text{F}-\text{B}-\text{F} \\ | \\ \text{F} \end{array}\right]^-$$

I   II   III   IV
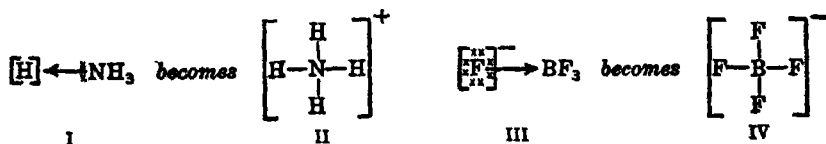
Rule of Covalence Maxima. An expression of the valence of an atom in terms of the electronic theory must take into consideration its capacity to form chemical combinations through electrovalent and covalent linkages. The electrovalence of an atom is determined by the number of its valence electrons; for an atom which tends to acquire

electrons it is equal to the number of electrons it requires to attain a stable number, and for an atom which tends to lose electrons it is equal to the number of electrons in excess of a stable number. The normal covalence of an atom is also determined by the number of its valence electrons, but since additional covalent links may be formed by co-ordination, it might appear that the total covalence could vary through wide limits. On the basis of chemical evidence Sidgwick[4,5] has formulated the following values for the covalence maxima of the elements: 2 for hydrogen; 4 (eight shared electrons) for elements of the first short period, from lithium through fluorine; 6 (twelve shared electrons) for elements of the second short period, from sodium through chlorine, and the first long period, from potassium through bromine; 8 (sixteen shared electrons) for atoms of higher atomic number.

Electroaffinity of Hydrogen. It is important to recognize the singular position of hydrogen in the periodic table.[13] The kernel of hydrogen is a bare proton, and as a result it has a higher effective nuclear charge than any other atomic kernel.* The small mass and relatively large charge of the proton account for its extraordinary mobility and for its ability to penetrate the electronic shells of other atoms. Hydrogen acts as a strongly electronegative element through its tendency to acquire an additional electron and attain a stable group of two. It usually forms a single covalent bond but occasionally takes complete possession of an electron pair and forms the hydride anion. The apparent tendency

$$\text{H} \cdot \overset{\frown}{+} \cdot \text{X} \rightarrow \text{H:X} \qquad \text{H} \cdot \overset{\frown}{+} \cdot \text{Na} \rightarrow \text{Na}^+[:\text{H}]^-$$
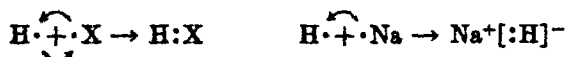
of hydrogen to act as an electropositive atom, as in the ionization of acids, is due to its great mobility. The concentration of *free* protons in the aqueous solution of a strong acid is extremely minute, and the ionization of acids must be regarded as the transfer of a hydrogen nucleus from one molecule to another, thus forming a complex ion.[13]

$$\text{H:X} + \text{H} \overset{..}{\underset{..}{\text{O}}} \text{:H} \rightarrow [\text{H} \overset{\overset{\text{H}}{..}}{\underset{..}{\text{O}}} \text{:H}]^+ \; [:\text{X}]^-$$
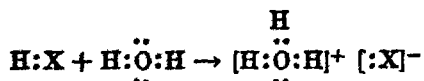
[13] Latimer and Rodebush, *J. Am. Chem. Soc.*, **42**, 1419 (1920); Rodebush, *Chem. Rev.*, **5**, 509 (1928); **19**, 59 (1936); see, also, Lowry, *J. Chem. Soc.*, **123**, 822 (1923); Huggins, *J. Org. Chem.*, **1**, 407 (1936); Lassettre, *Chem. Rev.*, **20**, 259 (1937); Rodebush and Buswell, *J. Phys. Chem.*, **43**, 219 (1939).

* This does not mean that hydrogen is the most electronegative element, since electronegativity is considered to represent the electron-attracting power of a *neutral atom* in a stable molecule (see p. 1855). On Pauling's scale of electronegativities hydrogen is assigned the value 2.1 and lies between boron (2.0) and carbon (2.5).

There is also good physical and chemical evidence that an atom of hydrogen can hold two other atoms together, as in the bifluoride ion, [F–H–F]⁻. The association of hydroxylic compounds, and formation of chelate rings (p. 1868) in certain *ortho*-substituted phenols and enolic forms of β-diketones, are further examples of 2-covalent hydrogen. Originally it was assumed that 2-covalent hydrogen held a group of four shared electrons, but this conception has been shown to be quite unlikely on physical grounds. The 2-covalent state of hydrogen is attributed to a condition of resonance between two structures, in the first of which the hydrogen is attached to one, and in the second to the other, of the two atoms which it holds together: A: H:B ⇌ A:H :B.

Resonance.[9] It is sometimes possible to write for a molecule, or polyatomic ion, two (or more) electronic formulas having the same arrangement of the atomic nuclei and differing merely in the positions assigned to the electrons in the system. If the electronic structures correspond to about the same energy content (and to the same number of unpaired electrons, if any), then neither electronic formula alone expresses the normal state of the molecule but instead a combination of both. The molecule will be more stable than either structure alone, as a result of resonance between the structures, and can be regarded as having a structure intermediate between the two (which cannot be expressed by the conventional symbols of structural chemistry). If two structures have the same energy the molecule resonates equally between the two; if one structure is much less stable than the other, its contribution to the normal state of the molecule will be relatively small and the molecule will be only slightly more stable than the more stable of the two structures (p. 1847).

In molecules that exhibit resonance the observed heat of formation is greater than the sum calculated from the heats of formation of the separate bonds, and the increased stability is interpreted as resonance energy.[14] It is found also that the distances between the atoms linked in a resonating system are somewhat smaller than the normal. These effects may be illustrated with carbon dioxide,[15] for which two different electronic formulas can be written.

$$\overset{+}{:}\!O\!\!=\!\!C\!\!-\!\!\overset{..}{\underset{..}{O}}\!: \ \rightleftarrows \ :\!\overset{..}{O}\!\!=\!\!C\!\!=\!\!\overset{..}{O}\!: \ \rightleftarrows \ :\!\overset{..}{O}\!\!-\!\!C\!\!=\!\!\overset{+}{O}\!:$$
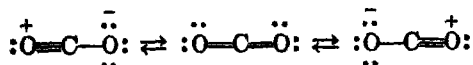
The essential conditions for resonance are satisfied, and since the calculated heats of formation of the two forms are nearly the same (348

[14] Pauling and collaborators, *J. Am. Chem. Soc.*, **53**, 3225 (1931); **54**, 996, 3570 (1932); *J. Chem. Phys.*, **1**, 362, 606, 679, 731 (1933).

[15] Sidgwick, *Ann. Repts. Chem. Soc. (London)*, **31**, 37 (1934).

kcal./mole for the symmetrical formula and about 350 for the other) they would contribute about equally to the normal state of the system. The effects of resonance are indicated by the following experimental observations: the observed heat of formation of carbon dioxide (380 kcal./mole) is about 32 kcal. greater than the value calculated for either one of the formulas; the observed distance between the terminal oxygen atoms is about 10 per cent less than the calculated values; and the chemical reactivity of the carbonyl groups is reduced far below that of aldehydes and ketones.

The theory of resonance is important in organic chemistry in accounting for the stability of systems which might be expected, on the basis of their structural formulas, to be more reactive than they actually are. Further examples are afforded by benzene (and aromatic systems in general), nitro compounds, isocyanates, amides, carboxylic acids, esters, and anions derived from carboxylic acids or enolic forms of carbonyl compounds (see Table V, p. 1837, and Table XIX, p. 1913).

The phenomenon of resonance involves merely a fluctuation of electrons without change of any atomic nucleus and is not to be confused with dynamic isomerism (tautomerism), which requires the displacement of a proton. In resonance, the time of change (if a change is considered to occur) is of the order of 10^{-15} second, but a mixture of two forms in tautomeric equilibrium would change very much more slowly.

ELECTRONIC CONFIGURATIONS OF ORGANIC MOLECULES

Derivation of Electronic Formulas. The electronic configurations of organic molecules containing only single bonds can be deduced directly from the traditional structural formulas merely by taking into account the number of valence electrons of the atoms concerned, the formation of electron pairs, and the tendency of the principal atoms (C, N, O, S, and the halogens) to form octets. However, double bonds of the conventional formulas may represent two different electronic systems: a true covalent double bond made up of four shared electrons or a coördinate link (semi-ionic double bond) of only two shared electrons.

Where more than one electronic structure is possible, the normal structure may be chosen by using the rule of covalence maxima and the principle of minimum residual charges formulated by Langmuir:[16] "The residual charge on each atom and on each group of atoms tends to a minimum." For this purpose fractional residual charges arising from an unequal distribution of the electron pair of a covalent bond (inductive displacements, p. 1842) can be neglected and the approximate residual

[16] Langmuir, *Science*, **54**, 59 (1921).

charge is given by the formula $E - (V_e + V_0)$, where E is the effective nuclear charge of the atom, V_e the number of electron pairs shared with other atoms (covalences), and V_0 the number of unshared electrons in its valence shell in the compound. In other words, if the sum of the covalences and the unshared electrons for an atom in a compound is greater or less than its effective nuclear charge, the atom bears a residual negative or positive charge.[12]

The use of these rules in deducing electronic formulas may be illustrated with two typical compounds, acetone and nitromethane. In the case of acetone, four possible structures might be considered for the carbonyl group. Since only the first of these satisfies the octet rule and the principle of minimum residual charges, this one may be taken to represent the normal electronic structure. The others may be used to indicate activated states of the molecule arising from dynamic electronic displacements (electromeric effects, p. 1845).
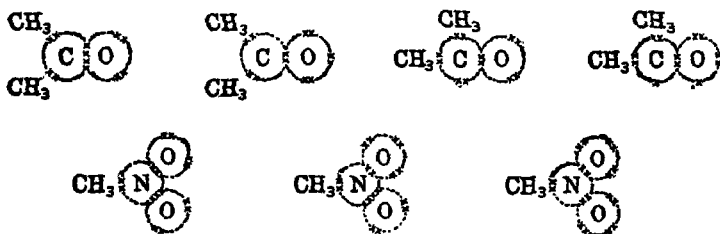
The structure of the nitro compounds offers a more difficult problem. The first formula leaves each atom with no residual charge but violates the octet rule. Although valence shells of more than eight electrons may occur with atoms beyond neon (atomic number, 10) there is strong evidence that the covalence maximum of four is never exceeded in atoms of the first period. In the second formula the octet rule is observed; in this structure the nitrogen has a residual charge of $+1$, the 1-covalent oxygen -1, and the 2-covalent oxygen ±0. In the third structure the nitrogen has only a sextet of electrons; its residual atomic charge is $+2$ and that of each oxygen is -1. Although it is possible that nitrogen may sometimes hold a stable group of six electrons, the principle of minimum residual charges indicates that the second formula will represent the normal state. Values of the electric moments of aromatic nitro compounds point to a symmetrical structure for the nitro group, but this can be explained on the basis of a mobile pair of electrons shifting back and forth between two oxygen atoms (resonance). The situation is analogous to the oscillating double bonds in the Kekulé formula for benzene. Problems of electronic configura-

tions in organic molecules have been attacked from the physical side by various means, such as stereochemistry, spectroscopic data, heats of combustion, electric moments, and the parachor (see Chapter 23, pp. 1744–1804).

Electronic Symbols. Owing to the complexity of expanded electronic formulas for organic molecules, various conventions have been introduced to simplify the representation of organic structures on the basis of the electronic theory. The usual symbol for an atom is used to designate the atomic kernel, that is, the atom without its valence electrons. The traditional bond of organic chemistry, either expressed or implied, is used with a precise significance to indicate a shared electron pair (covalence). A coördinate link, or semi-ionic double bond, may be shown by changing the covalent bond to an arrow pointing from the electron donor to the acceptor $(A \rightarrow B)$ or by using the conventional bond and indicating the resulting charges by plus and minus signs $(\overset{+}{A}\text{—}\overset{-}{B})$.

Unshared electron pairs need not be designated explicitly, since their presence is sufficiently obvious from the number of covalent bonds if the valence shell of the atom is complete. An unpaired electron in neutral atoms or free radicals may be indicated by a small dot or by the symbol e ($R\cdot$ or $R\text{-}e$). Ions need no symbol beyond the usual $+$ or $-$ sign, but it is frequently convenient to use brackets [ ] to delimit a polyatomic ion. The use of these conventions is illustrated by means of specific examples in the accompanying table.

Ionic Links. It will be observed that the condensed electronic formulas differ from conventional structural formulas only when ionic or semi-ionic (coördinate) links are present. In the ammonium and diazonium compounds the fact that one of the valences of pentavalent nitrogen is an electrovalence and differs from the other four is well established. Stereochemical evidence [17] (p. 413) shows that the four covalences of nitrogen have a tetrahedral arrangement * but the group held by the electrovalence does not have a fixed direction in space and is attracted electrostatically by the ammonium ion as a whole.

Since nitrogen cannot exceed a covalence of four it might be inferred that an ammonium base or salt could not exist in an undissociated form.

[17] Mills and Warren, *J. Chem. Soc.*, **127**, 2507 (1925); see Sidgwick (references 4 and 5, and also *Proc. Roy. Soc.*, **A 176**, 153 [1940]) for a general discussion of the space distribution of covalences.

* The tetrahedral arrangement appears to be the only space distribution for elements whose maximum covalence is four, and is the normal arrangement for many 4-covalent atoms of the higher periods (silicon, tin, phosphorus, arsenic, sulfur, selenium, tellurium, etc.). A plane arrangement appears to occur in 4-covalent nickel, palladium, and platinum; an octahedral arrangement occurs in all the 6-covalent compounds that have been examined (aluminum, chromium, iron, cobalt, nickel, platinum, etc.).

TABLE IV

ELECTRONIC CONFIGURATIONS OF TYPICAL ORGANIC COMPOUNDS

Conventional Formula	Electronic Formula	Condensed Electronic Formula

The table shows rows of chemical structures in three columns (Conventional Formula, Electronic Formula, Condensed Electronic Formula):

Row 1: $CH_2{=}CH_2$

Row 2: $CH_3{-}\overset{O}{\underset{}{C}}{-}CH_3$ (acetone)

Row 3: trimethylammonium chloride structure $\left[(CH_3)_2N H\cdots \right]^+ Cl^-$

Row 4: $CH_3{-}N({=}O)(CH_3)$ (trimethylamine oxide)

Row 5: $CH_3{-}N{=}O$ with O (nitroso/nitro)

Row 6: $CH_3{-}\overset{O}{\underset{O}{S}}{-}CH_3$ (dimethyl sulfone)

Row 7: $[C_6H_5{-}N{\equiv}N]^+Cl^-$

Row 8: triphenylmethyl $C_6H_5{-}\overset{C_6H_5}{\underset{C_6H_5}{C}}$

Row 9: $Na^+[:CH_2{-}C_6H_5]^-$

This inference is correct only for the quaternary ammonium compounds, since an undissociated form of the other ammonium compounds can arise by the formation of a coördinate link involving 2-covalent hydrogen.[18] An unionized amine-hydrate may be produced by coördination of a covalent hydrogen of water with the nitrogen atom of the amine.

$$(CH_3)_3N: + H{-}OH \rightleftarrows (CH_3)_3N \rightarrow H{-}OH \rightleftarrows [(CH_3)_3N{-}H]^+OH^-$$
<div align="center">Amine-hydrate</div>
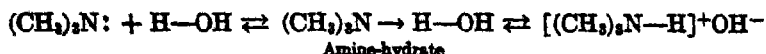
The stability of the undissociated form is due to the resonance effect associated with a hydrogen bond.*

The tertiary phosphines, arsines, and stibines give rise to quaternary cations strictly analogous to the ammonium cation. In the oxonium and sulfonium compounds, oxygen and sulfur have a covalence of three and one electrovalence; no compounds are known with more than three organic groups attached to oxygen or sulfur. Corresponding cations derived from the halogens occur only with iodine, in the diaryliodonium salts $[C_6H_5{-}I{-}C_6H_5]^+X^-$.

Oxonium salts of the type $[ROH_2]^+X^-$ and $[R_2OH]^+X^-$ are much less stable than the corresponding ammonium compounds. The interaction of an alcohol and a halogen acid can be represented by the following equilibrium, which is analogous to that of an amine and water:

$$\overset{\textstyle H}{\underset{\textstyle |}{}}$$
$$R{-}OH + H{-}X \rightleftarrows R{-}O \rightarrow H{-}X \rightleftarrows [R{-}OH_2]^+X^-$$
<div align="center">Unionized   Oxonium salt
complex</div>
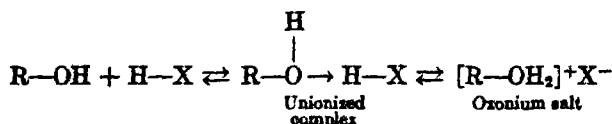
The most stable types of oxonium compounds are the cyclic structures derived from the pyrones. The enhanced stability of the pyrylium and pyroxonium salts may be attributed to an ability of the conjugated unsaturated system to dissipate the high residual charge on the oxygen atom by means of resonance effects.
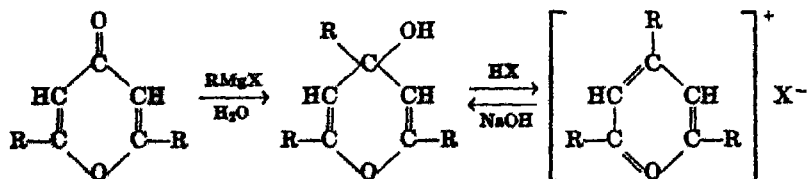
Organic anions occur commonly in the alkali metal salts of carboxylic acids, phenols and enolic forms of β-dicarbonyl compounds, oximes, and

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* The term "hydrogen bond" has been used frequently to indicate the unique bonding of 2-covalent hydrogen, but this expression is ambiguous and the term "hydrogen-bridge" seems preferable; see Huggins, *J. Org. Chem.*, **1**, 409 (1936).

aci-nitro compounds, and in the organometallic compounds of the alkali metals. It will be observed that all these compounds give rise to structures in which the stability of the anion may be increased through resonance effects, and that the formation of simple ionized salts is re-

TABLE V

RESONATING STRUCTURES OF ORGANIC ANIONS

Enolate anion of a β-diketone

Oximate anion   *aci*-Nitro anion

Benzyl anion   *o*-Quinonoid form   *p*-Quinonoid form
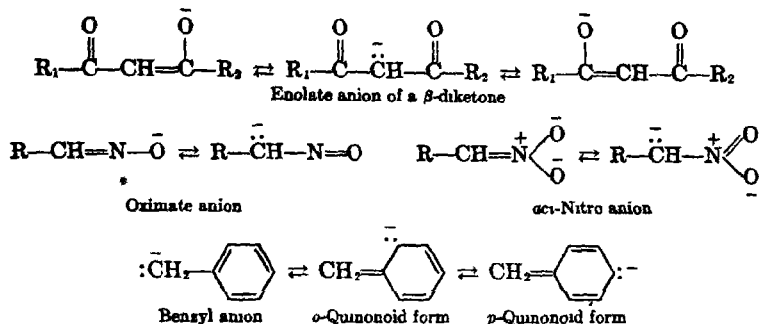
stricted largely to the alkali metals. Organic derivatives of the less active metals (such as beryllium, magnesium, and zinc) show a marked tendency to form coördination complexes of the Grignard type or to produce chelate ring structures (p. 1868) by intramolecular coördination.

Expanded Valence Shells. Although valence shells of groups of ten or twelve electrons may occur with elements of higher atomic number, experimental evidence indicates that the elements lying between helium and neon cannot expand their valence shells beyond an octet. The hypothesis that the elements in the first period do not expand their valence octets, even in the transitory coördination complexes that arise in the course of chemical reactions, is of considerable value in correlating the reactivity of atoms with their position in the periodic table.

All efforts to obtain derivatives of 5-covalent nitrogen have been unsuccessful. Nitrogen compounds containing five hydrocarbon groups, such as tetramethylammonium benzyl, were prepared by Schlenk [18] and found to behave as ionized salts, $[(CH_3)_4N]^+[:R]^-$ (p. 529). Attempts to obtain compounds with five simple alkyl groups attached to nitrogen, by the interaction of quaternary ammonium halides and metal alkyls, were also fruitless. [19] The products of the reactions indicate that the alkyl group derived from the metal alkyl does not enter the valence shell of the nitrogen atom. Similar experiments with quaternary halides of

[18] Schlenk and Holtz, *Ber.*, **49**, 603 (1916); **50**, 274 (1917).

[19] Marvel and collaborators, *J. Am. Chem. Soc.*, **48**, 2689 (1926); **49**, 2323 (1927); **51**, 3496 (1929); **52**, 376 (1930).

the phosphonium and arsonium type indicate that even these atoms do not expand their valence shells beyond an octet to hold a fifth alkyl group (pp. 425–426).[19] However, the existence of 5-covalent halides of the type R_3PCl_2 and $RAsCl_4$ shows that these atoms can hold a group of ten electrons (decet) when attached to highly electronegative elements.

The marked difference in the mode of decomposition of the quaternary ammonium bases [20] from that of the corresponding phosphonium, arsonium, and stibonium bases can be accounted for by the assumption that nitrogen is unable to hold a decet of electrons, even as an unstable intermediate state in the course of reaction.

$$[(C_2H_5)_3N-CH_2-CH_3]^+[OH]^- \rightarrow (C_2H_5)_3\overset{+}{N}-CH_2-CH_2-H\leftarrow OH$$

$$\rightarrow H-OH + (C_2H_5)_3\overset{+}{N}-CH_2-\overset{-}{C}H_2: \rightarrow (C_2H_5)_3N: + CH_2=CH_2$$
<div align="center">Olefinic decomposition</div>

$$[(C_2H_5)_3P-CH_2-CH_3]^+ [OH]^- \rightarrow (C_2H_5)_3P\begin{matrix} CH_2-CH_3 \\ OH \end{matrix}$$

$$\rightarrow (C_2H_5)_3P\begin{matrix} CH_2-CH_3 \\ O-H \end{matrix} \rightarrow (C_2H_5)_3\overset{+}{P}-\overset{-}{O} + CH_3-CH_3$$
<div align="center">Paraffinic decomposition</div>
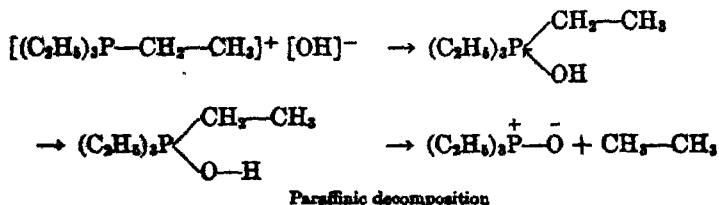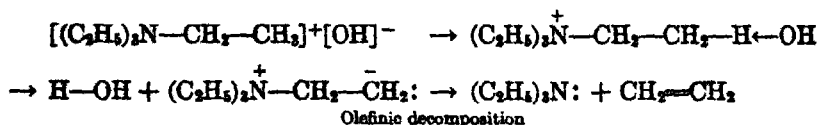
In a quaternary ammonium base the 4-covalent nitrogen cannot furnish a seat for the donor reagent (hydroxyl ion), and the attack occurs through acceptor activity conferred upon a hydrogen atom in the β-position of one of the alkyl groups. Elimination of water leaves the carbon in the β-position with an unshared electron pair and a high residual negative charge; the unshared electron pair is drawn toward the center of high positive charge, and the system breaks up into two more stable configurations, a tertiary amine and an olefin. In the quaternary phosphonium base the central atom is able, by expanding its valence shell, to act as an acceptor for the hydroxyl ion. Subsequent transformations of this complex result from the tendency of the central atom to revert to an octet. Expulsion of the hydroxyl ion merely reverses the original coordination, but the combination of an incipient alkyl anion with a proton from the hydroxyl group within the complex gives an irreversible decomposition into the tertiary phosphine-oxide and a paraffin.

Typical elements of the sixth group (sulfur, selenium, and tellurium) form ionized salts of the type $[R_3S:]^+X^-$ and show little tendency to expand the valence shell to a group of ten. In general, the formation of

[19] Ingold and collaborators, *J. Chem. Soc.*, 997 (1927); 3125, 3127 (1928); 2338, 2342, 2357 (1929); 705, 706, 713 (1930). *Cf. Ann. Repts. Chem. Soc. (London)*, **27**, 143 (1930).

an expanded valence shell occurs more readily with atoms of higher atomic number, and a group of twelve appears to be more stable than a decet. Tellurium, for example, forms a complex anion $[CH_3\text{—}\overset{..}{Te}I_4]^-$, in which it has five covalent bonds and an unshared electron pair. Although an expanded valence shell of ten or twelve electrons might occur in organic derivatives of sulfur (sulfinic acids, sulfoxides, sulfonic acids, sulfones, etc.), there is definite evidence from measurements of para-

I   II   III   IV
Sulfinic derivatives   Sulfonic derivatives
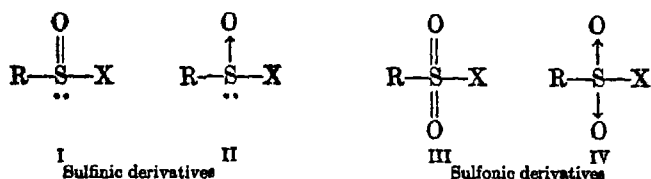
chors [10] and dipole moments that the octet structures (II and IV) represent the true configurations in these compounds.

The cleavage of sulfones by alkalies [20] gives evidence of the reluctance of sulfur to expand its valence shell but indicates that it can do so under favorable conditions. The dialkyl sulfones yield an olefin and an alkyl sulfinate; this reaction indicates the direct removal of a proton from the β-position and is strictly analogous to the decomposition of quaternary ammonium hydroxides. In the diaryl sulfones the olefinic decomposition is inhibited and the reaction is analogous to that of quaternary phosphonium hydroxides, which involves a temporary expansion of the valence shell.
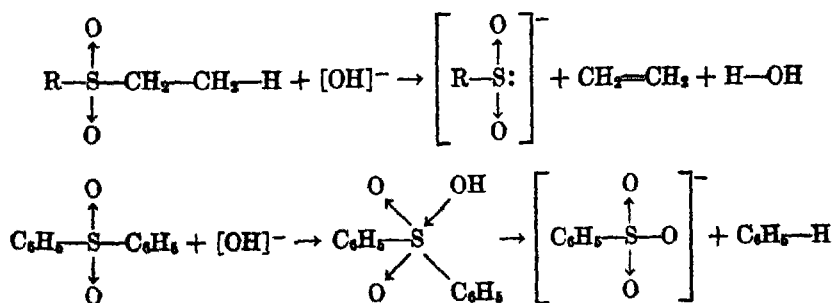
Covalent organic halides in which a halogen atom exerts a covalence greater than one appear to occur only with iodine, and particularly in the aryl iodides. At low temperatures methyl iodide forms a solid dichloride, $CH_3\text{—}ICl_2$, which decomposes into methyl chloride and iodine monochloride on warming to $-30°$. The unsaturated alkyl

iodides and aryl iodides form much more stable dichlorides, and the aryl compounds yield a series of derivatives containing 2-covalent iodine

$$CH_3-I + Cl_2 \xrightarrow{-80°} CH_3-I \leftarrow Cl-Cl \xrightarrow[\text{shift}]{\alpha,\gamma} I-Cl + CH_3-Cl$$

$$C_6H_5-I + Cl_2 \rightarrow C_6H_5-I \leftarrow Cl-Cl \longrightarrow [C_6H_5-I-Cl]^+Cl^-$$
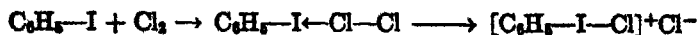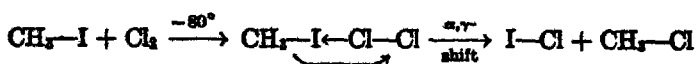
(iodosobenzene and diphenyliodonium salts) and 3-covalent iodine (iodoxybenzene).

The formation of the 2- and 3-covalent iodine compounds and their unusual reactions [21] can be interpreted upon the assumption that iodine in the link I-Aryl is capable of holding temporarily a decet of electrons but shows a strong tendency to revert to an octet. The structures of the stable derivatives involve only valence octets. A similar mechanism

$$\cdot [C_6H_5-I-Cl]^+ \xrightarrow{[OH]^-} C_6H_5-I \leftarrow OH \rightarrow C_6H_5-\overset{+}{I}-\overset{-}{O} + HCl$$
$$\text{Iodosobenzene}$$

$$2R-IO \rightarrow R-\overset{\overset{-}{O}}{\underset{}{I}} \leftarrow O-\overset{+}{I}-R \rightarrow R-\overset{\overset{O}{}}{\underset{++}{I}}-\overset{-}{O} + R-I$$
$$\text{Iodoxybenzene}$$

$$R-IO_2 + R-IO \rightarrow R-\overset{+}{I}-O-\overset{\overset{-}{O}}{\underset{\underset{R}{}}{\overset{|+}{I}}}-O^- \xrightarrow[\text{shift}]{\alpha,\gamma} [R-I-R]^+ [IO_3]^-$$
$$\text{Diphenyliodonium salt}$$
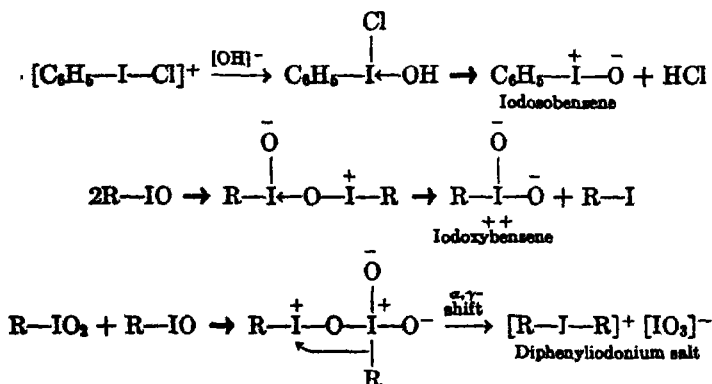
may be applied to certain reactions of the alkyl halides, and particularly to anomalous reactions of the iodides, since the tendency to expand the valence shell follows the sequence: iodine > bromine > chlorine.

CLASSIFICATION OF ELECTRON DISPLACEMENTS

In the development of current theories dealing with the electronic mechanism of organic reactions by Robinson [6] and by Ingold,[7] the activation of a molecule is considered to arise largely through active or incipient electron displacements leading to the development of a center of high or low electron density. Chemical change is pictured as an electrical transaction, and molecules are considered to react by virtue of a constitutional affinity either for electrons (electrophiles) or for atomic nuclei (nucleophiles). When an electron-seeking reagent attacks some

[21] For a discussion of reactions of the iodoxy group (—IO₄) see Masson, Race, and Pounder, J. Chem. Soc., 1669 (1935).

center in an organic molecule, reaction will take place if the center is able to supply electrons to the requisite extent; the development of a critical electron density at the site of reaction is an essential feature of the development of the energy of activation. Thus, the mechanism of supplying electrons to the reaction zone becomes the main consideration; the more readily the necessary electron density can be furnished, the more the reaction will be facilitated. For a nucleophilic reagent the primary necessity is a center of low electron density at the site of reaction, and groups which withdraw electrons from the reaction zone will facilitate the reaction.

The molecular model which serves as a basis for the modern electrochemical (electronic) theories of reactions is one that visualizes a space distribution of atomic nuclei and electrons (as point charges) maintained by elastic forces about fixed relative positions.[7] This simple picture has been elaborated, to the dismay and confusion of many organic chemists, by the introduction of wave-mechanical ideas of a continuous statistical distribution of electron density, of quantized states, and of resonance (degeneracy). However, the more complicated picture has served, on the whole, merely to correlate and place upon a more definite physical basis a variety of phenomena that were long recognized by organic chemists.

The simpler or the more complex picture leads to the view that the electrical specification of a molecule requires a knowledge of two kinds of electrical quantities. These are concerned with the positions and the mobility of the charges, that is, with the state of polarization of the system *and* with its polarizability. Polarizability represents an intrinsic susceptibility to polarization, a deformability, which becomes operative under the influence of the environment.

The extent to which a given group in an organic molecule can contribute to the activation for reaction involves considerations of the polarization and the polarizability of the group, and of the electrical requirements for the particular reaction. The general acceptance of electronic theories by organic chemists has been delayed by the use of ill-defined conceptions of "polarity," "electron attraction," and "relative electronegativities" (p. 1854) along with an insufficient appreciation of the duplex mechanism of activation and of the contribution of the environment (reagents, solvent media, catalysts, etc.). The circumstance that various reactions, intended to measure relative "polarity" or "electronegativity," do not place groups in an identical sequence is to be expected: polarization and polarizability are independent variables, and their relative contributions vary with the nature of the reaction and of the environment.[7]

The activation of a molecule is considered to involve two forms of electron displacement. Inductive displacements, designated as *I* effects, arise mainly from an unequal extent of sharing of the electron pair of a covalent bond and affect the state of polarization of the atoms in the link; these effects represent a relatively permanent condition of the molecule. Electromeric (dynamic) displacements, designated as *T* or *E* effects, are associated with unshared electron pairs or multiple covalent bonds and concern primarily the polarizability of the structure; electromeric effects are much more time-variable than inductive effects.

Ingold's elaboration of the theory has led to the postulation of four polar effects, as indicated in the following scheme:

Electronic Mechanism *	Electrical Classification	
	Polarization (permanent)	Polarizability (dynamic)
General inductive (*I*) symbol ⟶	Inductive (I_s)	Inductomeric (I_d)
Tautomeric (*T*) symbol ⌒	Mesomeric (*M*)	Electromeric (*E*)

* The notations I_s and I_d were not used by Ingold but are introduced here to avoid ambiguity in referring to this scheme.

In the present state of the knowledge concerning polar effects, the validity of this analysis or its practical utility is not unquestioned. It is convenient, frequently, to indicate the general mechanisms (*I* and *T* effects) without further reference to permanent or dynamic factors.

The following paragraphs are devoted to definitions of the terms used currently in the application of the theory of electron displacements in organic reactions. It is essential to have a clear conception of the precise usage of the terms, and to recognize the significance and the limitations of the different electronic effects. Furthermore, two different effects may be present in the same bond and they may reinforce or oppose each other. In the system C—OH, there is an inductive displacement toward the hydroxyl group *and* an electromeric effect in the opposite direction. Each effect can act independently, and the contribution in a given reaction requires a consideration of several factors, especially the electrical demand of the reagent.

General Inductive Effects. Lewis pointed out that the electron pair of a covalent bond may be shared by the two atomic kernels in such a way that there is no *permanent* polarization (as in the symmetrical

links H_3C—CH_3, H_2N—NH_2, and Cl—Cl), or the binding pair may be shifted toward one atom so as to give that atom a fractional negative charge and the other atom a corresponding positive charge (as in the unsymmetrical bonds H_3C—NH_2, H_3C—OH, H_3C—Cl). The term inductive effect is used to designate a permanent displacement (polarization) in which the electron pair remains within the valence shell of both atoms. This displacement is restricted by the fundamental principle requiring the maintenance of stable electronic configurations (especially the octet rule) and is not regarded as sufficient *in itself* to produce a reactive molecule. Inductive effects are considered to act largely through enhancing or restraining electromeric effects. Experimental evidence indicates that I effects diminish rapidly in a saturated chain and become negligible beyond two or three atoms.

The direction of inductive effects is considered usually in a relative sense *with reference to hydrogen*, that is, from the standpoint of relative influences of substituents in a given system. A group X would be considered to exert an effect of electron release in the compound X—CR_3 if the electron density in the residue —CR_3 were greater in this compound than in H—CR_3. Similarly, the group Y is classified as electron-

$$X \rightarrow CR_3 \qquad H—CR_3 \qquad Y \leftarrow CR_3$$

| $-I$ effect (Electron release) | Reference standard | $+I$ effect (Electron attraction) |

attracting in Y—CR_3, if the electron density in —CR_3 is less in this compound than in H—CR_3. Electron release is distinguished by a negative sign and electron attraction by a positive sign, so that they may be indicated by the symbols $-I$ and $+I$ (Robinson).* In structural formulas the direction of electronic displacement may be indicated by an arrow head placed at the *center* of the bond (not to be confused with the symbol for a coördinate link).

The influence of substituents on the dissociation of organic acids affords a simple illustration of inductive effects, and suitable comparisons give information as to the relative effects of various atoms and groups. The ionization of acetic acid involves the detachment of a proton from the carboxyl group by means of a solvent molecule, and this process will be governed (in a given solvent) by the degree of attraction of the acetoxyl group for the proton.

$$CH_3—CO_2—H + OH_2 \rightleftarrows [CH_3—CO_2]^- + [H—OH_2]^+$$
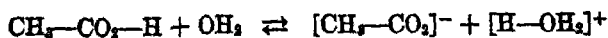
* In the present discussion the signs attached to I and E effects are the reverse of those employed by Ingold, although the directions of the effects are considered to be the same. The signs used by Ingold indicate the effect of the displacement upon the groups X or Y, rather than upon —CR_3.

When a substituent is introduced into the acid, the influence of the substituent relative to that of hydrogen is expressed by the extent to which the equilibrium is displaced in one direction or the other. If the substituent withdraws electrons ($+I$ effect) the proton will be held less

$$X\text{---}\!\leftarrow\!\text{CH}_2\text{---}\!\leftarrow\!\text{CO}_2\text{---}\!\leftarrow\!\text{H} \qquad\qquad Y\text{---}\!\rightarrow\!\text{CH}_2\text{---}\!\rightarrow\!\text{CO}_2\text{---}\!\rightarrow\!\text{H}$$

Increased acidity   Diminished acidity

firmly in the carboxyl group and the acidic strength will be increased; if the substituent releases electrons ($-I$ effect) the resulting increase in electron density in the carboxyl group will hold the proton more strongly and the acidic strength will be diminished.[22] The constants given in Table VI show that all the common substituents except alkyl groups exert a $+I$ effect, and the order is as follows:

$$F > Cl > Br > I > OCH_3 > NHCOCH_3 > C_6H_5 > CH\!=\!CH_2 > H > CH_3$$

TABLE VI

DISSOCIATION CONSTANTS OF SUBSTITUTED ACETIC ACIDS

Substituent	$K \times 10^5$	Substituent	$K \times 10^5$
—CH$_3$	1.4	—OCH$_3$	33
—H	1.8	—I	75
—CH=CH$_2$	4.5	—Br	138
—C$_6$H$_5$	5.5	—Cl	155
—NHCOCH$_3$	22.5	—F	210

Owing to the use of an arbitrary reference standard, an effect of electron release relative to hydrogen does not imply that the group X *necessarily* becomes the positive end of an electrical dipole, nor that the atoms in the link $X \rightarrow\!\!-\! C$ bear residual atomic charges of opposite sign ("alternating polarity"). Likewise, electron attraction relative to hydrogen does not imply that the group Y becomes the negative end of a dipole. This is shown by a consideration of some specific examples.

$$:\overset{\,-}{\underset{\,\cdot\cdot}{O}}\!\rightarrow\!\!-\!\overset{\delta-}{CR_3} \qquad\qquad :\overset{\delta-}{\underset{\,\cdot\cdot}{Cl}}\!-\!\!\leftarrow\!\overset{\delta+}{CR_3} \qquad\qquad \overset{+}{H_3N}\!-\!\!\leftarrow\!\overset{\delta+}{CR_3}$$
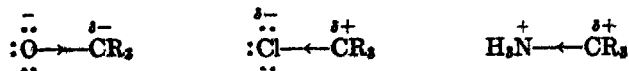
In an alkoxide anion the strong $-I$ effect of the anionic center results in the transfer of a small fractional negative charge (symbol $\delta-$) to the attached carbon atom, and in the alkylammonium cation the strong

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[22] See Dippy, *Chem. Rev.*, **25**, 167 (1939), for a discussion of the influence of substituents on the dissociation constants of organic acids.

+*I* effect of the cationic center leaves the attached carbon with a small residual positive charge (symbol δ+).* In both cases the sign of the induced charge is the same as that of the polar group itself. With an electrically neutral substituent such as chlorine, the +*I* effect of the halogen atom does actually create an electrical dipole, and the atoms in this link bear fractional charges of opposite sign.

A summary based upon Ingold's classification of the inductive effects of a number of organic groups, and their relative magnitudes, is given in Table VII.

TABLE VII

INDUCTIVE EFFECTS

Electron release (−*I*)

$$\overset{-}{-NR} > \overset{-}{-O} > \overset{-}{-S} > \overset{-}{-Se}$$
$$-Li > -MgX > -ZnX > -CdX > -HgX$$
$$-AlR_2 > -SiR_3 > -SnR_3 > -PbR_3$$
$$-C(CH_3)_3 > -CH(CH_3)_2 > -CH_2-CH_3 > -CH_3$$

Electron attraction (+*I*)

$\overset{+}{-OR_2} > \overset{+}{-NR_3} > \overset{+}{-PR_3} > -AsR_3$	$\overset{+}{-NR_3} > -NR_2; \ \overset{+}{-OR_2} > -OR$
$\overset{+}{-OR_2} > \overset{+}{-SR_2} > \overset{+}{-SeR_2}$	$-F > -OR > -NR_2$
$\overset{+}{-NR_3} > -NO_2 > -AsO_3H_2$	$-F > -Cl > -Br > -I$
$-SO_2-R > -SO-R; \ -SO_2-R > -SO_3^-$	$-C{\equiv}C-R > -CR{=}CR_2$
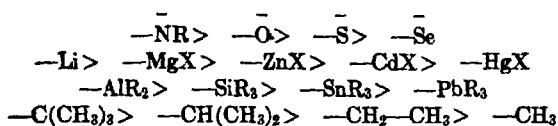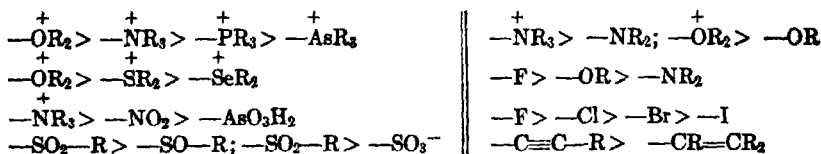
Electromeric Effects. Tautomeric displacements occur in systems containing double bonds or triple bonds, and in single bonds containing an atom that holds an unshared electron pair. In the first case, one

$$R-CH{=}O \qquad R-C{\equiv}N \qquad C_6H_5-NH_2 \qquad C_6H_5-OH$$

Dynamic electron withdrawal (+*E* or +*T* effect) Dynamic electron release (−*E* or −*T* effect)

atom tends to withdraw an electron pair from the multiple link and create an electronic deficit in the valence shell of the other atom. In the second, an unshared electron pair is released toward the adjacent atom so that the covalence of the link tends to increase.

The symbol R—CH꠹O implies that to some unknown extent the electrons of the double bond are breaking away from the carbon atom

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* To avoid confusion the symbols + and − are restricted to the designation of formal (integral) charges of ions or ionic centers. The symbols δ+ and δ− are used to indicate fractional charges acquired through electron displacements.

and remaining attached to oxygen. The situation may be pictured from the standpoint of electronic configurations in the following way:

$$R\text{—}CH\text{=}O \quad \rightleftarrows \quad R\text{—}CH\overset{\frown}{\text{=}}O \quad \rightleftarrows \quad R\text{—}CH\text{—}\overset{\cdot\cdot}{\underset{\cdot\cdot}{O}}:$$

Normal state   Activated state
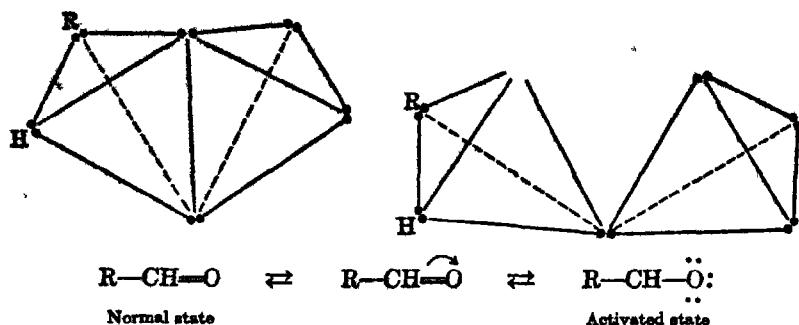
The symbol $C_6H_5\overset{\frown}{\text{—}}OH$ implies that an electron pair of the oxygen atom can be available for the formation of a double bond with the adjacent carbon atom. Owing to the inability of carbon to expand its valence shell, the effect of electron release by oxygen can occur only in the course of a process involving the simultaneous release of an electron pair by the carbon atom. In the case of phenol, the $-T$ effect of the hydroxyl group will facilitate the development of a center of high electron density (donor center), at an *ortho-* or *para-*position of the benzene ring.

ortho-Activation   para-Activation
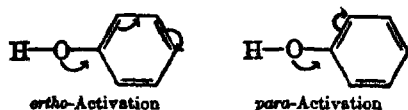
Consequently the $-T$ effect will facilitate attack of the aromatic nucleus, at an *ortho-* or *para-*position, *by an electron-seeking reagent.* The $-I$ effect in the link $C\rightarrow OH$ does not contribute directly to this kind of reaction since it diminishes the electron density in the aromatic nucleus; it can serve merely to increase the proton-escaping tendency of C—H bonds in the aromatic system. The effects of substituents such as —NH$_2$ and halogens are similar qualitatively to the —OH group.

Electromeric displacements of either sign ($+T$ and $-T$ effects) will tend to be restrained by the opposing influence of the electrical charges that arise concurrently, but other restrictions are more important. Electron withdrawal ($+T$) is held back mainly by the restoring force of the unstable electronic configuration (open sextet) of the deficient atom. Electron release ($-T$) is reversed by the opposing $+T$ effect of the double bond which it creates, but the main consideration is the ability of the attached atom to transmit the effect through one or

more multiple bonds (as in phenol or aniline). Even if the attached atom is capable of expanding its valence shell beyond an octet, which is possible for atoms beyond neon (see covalence maxima, p. 1829), the relatively unstable configuration of the expanded shell will have a strong opposing influence. Consequently, electromeric effects represent mainly an inherent ability of the system to undergo an effective electron displacement under the influence of an external polar center.

TABLE VIII

ELECTROMERIC POLARIZABILITIES (INGOLD)

$-E$ (*Electron release*)

$\overset{-}{—O}>$ —OR> $\overset{+}{—OR_2}$; etc.

—NR$_2$> —OR> —F; etc.

—I> —Br> —Cl> —F; —SCN; etc.

$+E$ (*Electron withdrawal*)

$\overset{+}{—C}=NR_2>$ —C=NR; etc.

—C=O> —C=NR; etc.

—COR> —COCl> —CO$_2$R> —CO—NR$_2$> —CO$\overset{-}{_2}$; etc.

$\pm E$ *type*

—C=C; —C=C—C=C; —C$_6$H$_5$; etc.

Polarizability effects are concerned with the mobility of the electronic system and are considered to have greater time-variability than inductive effects. Since chemical reaction is assumed to occur only in molecules in an exceptional (activated) state, the momentary surge of electron density associated with the dynamic effects is intimately bound up with the process of activation (p. 1862). The dynamic components (I_d and E) are considered to be capable of giving a direct impetus to one course of reaction but incapable of exerting a *direct* opposition to an alternative course.

Mesomeric Polarization (Resonance Effects). There is physical evidence that molecules containing two appropriately disposed electromeric systems of opposite types form a molecular dipole. The resulting internal compensation of the dynamic effects of electron release and electron withdrawal leads to a diminished activity of the system toward external donor and acceptor reagents. In the system illustrated, the permanent polarization reduces the basic strength of the amino group and depresses the reactivity of the carbonyl group. Robinson, beginning in 1926, developed a number of generalizations pertaining to struc-

$$R_2\overset{..}{N}—CH=CH—CH=O \rightleftarrows R_2\overset{+}{N}=CH—CH=CH—\overset{-}{O}$$
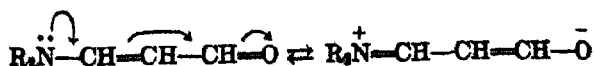
tures of this kind, which were classified later as "polyenoid," "hetero-enoid," "katio-enoid," and "neutralised" systems.* Examples of these structures and their characteristic behavior are discussed later (poly-functional electromeric systems, pp. 1908–1928).

A conception of "electronic strain," which is essentially the same as the resonance principle, was adumbrated by C. K. Ingold and E. H. Ingold in 1926. In its subsequent development the terms "mesomer-ism" and "tautomeric degeneracy" were introduced, and the permanent state of polarization associated with electromeric effects was designated as a mesomeric effect (symbol M).

The magnitude of the mesomeric effect in a given system such as (I) will depend upon the relative stability of the alternative structure (II). Mesomeric polarization of (I) requires that X increase its cova-lence by one unit and acquire a cationic charge, and the opposite change for Y. The presence of preëxisting electrical charges on X or Y will have

$$\overset{\frown}{X}-C \overset{\frown}{=} C - \overset{\frown}{C} = Y \rightleftarrows \overset{+}{X} = C - C = C - \overset{-}{Y}$$

$$\text{I} \qquad\qquad\qquad \text{II}$$
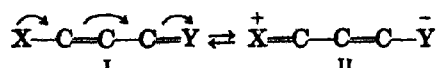
an important influence. Electron release by X will be facilitated by a negative charge, and suppressed by a positive charge. Obviously, elec-tron withdrawal by Y will be influenced in the opposite way by electrical charges.

TABLE IX

MESOMERIC EFFECTS *

$-M$ effect of X

(Electron release and increase of covalence)

$$-\overset{-}{CR_2} > \quad -\overset{-}{NR} > \quad -\overset{-}{O}$$

$$-\overset{-}{NR} > \quad -NR_2; \quad -\overset{-}{O} > \quad -OR > \quad -\overset{-}{OR_2}; \quad -\overset{-}{S} > \quad -SR > \quad -\overset{+}{SR_3}$$

$$-NR_2 > \quad -OR > \quad -F; \quad -SR > \quad -OR; \quad -F > \quad -Cl > \quad -Br > -I$$
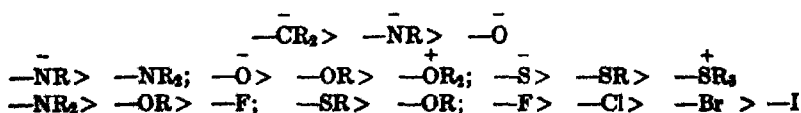
$+M$ effect of Y

(Electron attraction and decrease of covalence)

$$=\overset{+}{NR_2} > \quad =NR; \quad =S > \quad =O \text{ (e.g., in } -C=S \text{ and } -C=O)$$

$$=O > \quad =NR > \quad =CR_2 \text{ (e.g., in } -C=O, \quad -C=NR \text{ and } -N=O, \quad -N=NR)$$

$$\equiv N \text{ (e.g., in } -C\equiv N \text{ and } -N\equiv N)$$
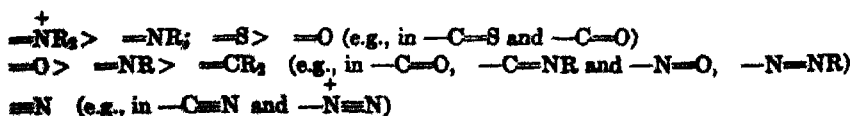
* In this table the $-M$ effect of the halogens is the reverse of that given in the first edition. The relative effect of the halogens has been the subject of much controversy owing to the circumstance that several factors are operative. For a concise discussion of the four distinct polar effects of the halogen atoms, see Bird and Ingold, *J. Chem. Soc.*, 927 (1938).

Inductomeric Polarizability. In 1933 Ingold introduced the term inductomeric polarizability to designate a polarizability effect arising in single bonds during the course of reaction by an inductive mechanism, that is, a polarizability effect associated with changes in the sharing of the bonding electron-pair under the influence of a reagent. This concept is the counterpart of the idea of a permanent polarization associated with an electromeric displacement (mesomeric effect).

Polarizability effects are due to the deformability of one molecular system under the influence of the polarizing field (polarization) of another. Thus, the close approach of an external polar center may alter the normal distribution of the electron pair of a covalent bond through the deforming action of its polarizing field. The response of a given system to this effect will depend upon the polarizability of its members (see bond polarizabilities, p. 1856).

$$A\!-\!\cdot\!-B \qquad\qquad [X]^- \overset{\delta+\ \ \delta-}{A\!-\!\!-\!\cdot\!B}$$

Normal (resting) state   Dynamic response to reagent

The contribution of inductive polarizability effects is of particular significance in the alkyl groups. These groups merely exert the polar effects which are impressed upon them by other groups in the molecule. The general inductive effect (relative to hydrogen) of CH_3-, and all saturated alkyl groups, is zero if the comparison is made between CH_3-CH_3 and CH_3-H; but CH_3- exerts a weak effect of electron release $(-I)$ if CH_3-CO_2H and $H-CO_2H$, or $CH_3-C_6H_5$ and $H-C_6H_5$, are compared. Since the common organic substituents $(-NH_2, -OH,$ halogens, etc.) have a stronger attraction for electrons than do alkyl groups, the latter will usually exert an effect of electron release. In combinations with groups of lower electron attraction, the opposite effect may be expected.

Alkyl groups are more polarizable than hydrogen, and in the course of reactions one may expect this property to result in a dynamic effect such as $CH_3-\!\!\leftarrow\!C$ relative to $H-C$. Ingold makes this statement:[7] "It is provisionally assumed that an inductomeric polarizability is the same for both directions; this would surely hold for small electron displacements, but it is unlikely to be more than roughly true for displacements of the magnitude of those which occur during reactions." Ingold's classification of the inductomeric polarizabilities of typical substituents is presented in the following tabulation.*

* For Fajans' generalizations relating to deformation and deformabilities of ions, see p. 1857. The "deformation rules" of Fajans may be used qualitatively to estimate the relative tendency of covalent bonds to undergo ionization *under comparable conditions,* and also to estimate the relative stability of organic ions.

<div align="center">± INDUCTOMERIC POLARIZABILITIES</div>

$$-\overset{-}{O}> \quad -OR> \quad -\overset{+}{O}R_2; \quad -NR_2> \quad -\overset{+}{N}R_3; \quad etc.$$
$$-CR_3> \quad -NR_2> \quad -OR> \quad -F; \quad etc.$$
$$-I> \quad -Br> \quad -Cl> \quad -F; \quad etc.$$
$$-CR_3> \quad -CHR_2> \quad -CH_2R> \quad -CH_3; \quad -CH_3> \quad -H; \quad etc.$$
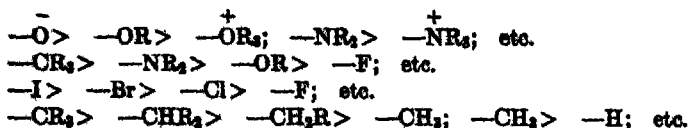
It is evident that there are certain special types of organic reactions which cannot be dealt with adequately on the basis of the four electronic effects outlined in the preceding paragraphs. When a reaction involves redistribution of atomic nuclei among themselves (tautomerism, and intramolecular rearrangements), the introduction of additional special principles is required. Furthermore, the two mechanisms of electronic displacement have been considered from the standpoint that all the electrons are paired, and cannot be applied without extension to molecules that contain an unpaired electron (free radicals). However, the marked tendency of unpaired electrons to form pairs (rule of two) and the anomalous properties of systems containing an unpaired electron (odd molecules) justify the assumption that the most characteristic reactions of covalent bonds involve retention of the binding pair by one atom of the link rather than fission into free radicals. The formation of free radicals and the interpretation of their chemical behavior will be considered elsewhere (p. 582 and pp. 1928–1934).

POLAR CHARACTERISTICS OF COVALENT BONDS

Residual Charges. In a symmetrical covalent link, A—A or B—B, the binding pair of electrons is distributed equally between the two atomic kernels, so that in the normal or average state there is no permanent polarization of the link. In an unsymmetrical covalent link A—B, the electron pair may be shifted toward one atom and away from the other, so that A and B acquire fractional charges ($\delta\pm$) and an electrical dipole is created. From estimations of the dipole moments of individual links and of the distances between the atomic nuclei, Sidgwick [5] has calculated the approximate extent of this displacement, or the inequality of sharing of the electron pair, in a number of the common covalent links. These values are shown in Table X, where the links are written with the positive end of the dipole at the left, and the symbol $\delta\pm$ expresses the residual charge as a fraction of the charge of an electron (4.77×10^{-10} e.s.u.).

The existence of a residual charge on the atoms of a covalent bond does not in itself give rise to a condition of instability. Pauling has de-

TABLE X

RESIDUAL CHARGES IN COVALENT LINKS (SIDGWICK)

Link $\delta^+ \quad \delta^-$ $\longrightarrow$	Moment ($\Delta E \times d$)	Internuclear Distance d, in Ångström Units	ΔE in Electrostatic Units	$\delta \pm$ Electron Charge Units
H—C	0 2	1.14	0.18	0.04
H—N	1.3	1.08	1.2	0.25
H—O	1.6	1.07	1.5	0.31
H—P	0 55	1.24	0.44	0.1
H—S	0.8	1.43	0.6	0.13
H—Cl	1.03	1.27	0 81	0.17
H—Br	0.78	1.41	0.55	0.12
H—I	0.38	1.61	0.24	0.05
C—N	0.4	1.48	0.3	0.06
C≡N	3.3	1.15	2.9	0.61
C—O	0.9	1.47	0 6	0.13
C=O	2.5	1.27	2.0	0.42
C—S	1.2	1.83	0.7	0.15
C=S	3 0	1.59	1.9	0.40
C—F	1.5	1.45	1.03	0.22
C—Cl	1 7	1.74	1.0	0.21
C—Br	1.6	1.90	0 8	0.17
C—I	1.4	2.12	0.7	0.15
N—O	0 5	1.41	0.4	0.08
N=O	1.9	1.21	1 6	0.34

veloped the view that a single bond may be described as resonating between the covalent extreme and the ionic extreme. If the extreme covalent structure A:B corresponds to the same bonding energy as the extreme ionic structure A$^+$:B$^-$, then the two structures will contribute equally to the actual state of the molecule, and the actual bond energy will be greater than the bond energy for either structure alone by an amount equal to the interaction of the two structures; that is, the molecule will be stabilized by resonance between the two structures. If one of the two extreme structures corresponds to a greater bond energy than the other, the more stable structure will contribute more to the actual state of the molecule than the less stable one, and the actual bond energy will be somewhat greater than that of the more stable structure. In dealing with the properties of single covalent bonds it is convenient, instead of referring to resonance between the extreme structures, to describe the bond as a *covalent bond with partial ionic character*. The ionic contribution is exceedingly small (less than 2 per cent) in symmet-

rical bonds such as H—H and Cl—Cl, and in bonds between atoms of similar electronegativity such as Br—Cl. The ionic contribution becomes greater when the atoms in combination are quite different in electronegativity.

The fact that atoms of a covalent bond bear fractional charges does not mean that there is no essential distinction between a covalent and an ionic bond, nor does it imply that a covalent molecule A—B exists in equilibrium with the ion pairs $A^+ :B^-$ and $A^- B^+$. Theoretical considerations and experimental evidence support the view that in the great majority of links the bond will be due almost entirely either to electron sharing (covalence) or to electrostatic forces (electrovalence). In typical single covalent links the inequality of sharing of the electron pair (partial ionic character) does not usually exceed 20 per cent ($\delta \pm 0.2$), and in typical ionic links the reduction in dipole moment resulting from mutual deformation of the ions is usually less than 20 per cent.

Bond Energies. The amount of energy evolved in the formation of a molecule A—B (in the gaseous state) from the two neutral atoms or radicals A· and ·B (in the gaseous state) is called the bond energy. The bond energy represents, conversely, the amount of energy required to dissociate a gaseous molecule of A—B into the neutral atoms or radicals A· and ·B. Empirical values of bond energies of simple diatomic molecules may be obtained from thermochemical or spectroscopic data, and average bond energies for individual bonds in polyatomic molecules may be calculated from heats of combustion, or heats of formation of the compounds, together with the heats of formation of the products of combustion (CO_2 and H_2O) and the heats of formation of the atoms from the elements in their standard states. The values of a number of bond energies compiled by Pauling [*] are given in Table XI; these values are for *actual bonds* with partial ionic character and not for extreme (ideal) covalent bonds, and are designed only for use with atoms having no formal electrical charge. For molecules that can be represented by one valence bond structure (non-resonating systems), these bond-energy values are additive and the sum gives a fairly good approximation to the heat of formation of the compound from the atoms. For molecules that can have more than one valence bond formula (resonating systems) the actual heat of formation as found experimentally is *greater* than the sum of the individual bond energies, and the difference represents the degree to which the molecule has been stabilized by resonance among the several formulas.

If an unsymmetrical covalent bond A—B were an average of the symmetrical bonds A—A and B—B, and no perturbing factors intervened, one would expect the value of the bond energy D(A—B) to be

TABLE XI

SINGLE BOND ENERGY VALUES (PAULING)
(In kilocalories per mole)

Symmetrical Bonds

H—H	103.4	P—P	18.9	F—F	63.5
C—C	58.6	As—As	15.1	Cl—Cl	57.8
Si—Si	42.5	O—O	34.9	Br—Br	46.1
Ge—Ge	42.5	S—S	63.8	I—I	36.2
N—N	20.0	Se—Se	57.6		

Unsymmetrical Bonds

C—H	87.3	C—S	54.5	P—Cl	62.8
Si—H	75.1	C—F	107 0	P—Br	49.2
N—H	83.7	C—Cl	66.5	P—I	35.2
P—H	63.0	C—Br	54.0	As—Cl	60.3
As—H	47 3	C—I	45 5	As—Br	48.0
O—H	110.2	Si—O	89 3	As—I	33.1
S—H	87.5	Si—S	60.9	O—F	58.6
Se—H	73.0	Si—F	143.0	O—Cl	49.3
H—F	147.5	Si—Cl	85 8	S—Cl	66.1
H—Cl	102.7	Si—Br	69.3	S—Br	57.2
H—Br	87 3	Si—I	51.1	Se—Cl	66.8
H—I	71.4	Ge—Cl	104 1	Cl—F	86.4
C—Si	57 6	N—F	68.8	Br—Cl	52.7
C—N	48.6	N—Cl	38 4	I—Cl	51.0
C—O	70.0	N—O *	57.0	I—Br	42.9

Multiple Bond Energy Values

C=C	100	C=N	94
C≡C	123	C≡N	144 (in HCN)
C=O	142 (in CH₂O)	C≡N	150 (in RCN)
C=O	149 (in RCHO)	C=S	103
C=O	152 (in RCOR)	N=O	113 *

* The values for N—O and N=O were obtained from the heats of combustion of ethyl nitrate, dimethylnitrosamine, acetoxime, and nitrosobenzene by making them consistent within themselves and with suitable resonance energies The value 57 kcal. for N—O includes the energy of the resonance X=N—OH ⇄ X̄—N=OH⁺; see Branch and Calvin, "The Theory of Organic Chemistry," Prentice-Hall, Inc., New York (1941).

equal to the arithmetic mean of the corresponding symmetrical bond energies $D(A—A)$ and $D(B—B)$. On the basis of the theory of ionic resonance in covalent bonds, Pauling [9] has postulated that the actual bond energy of an unsymmetrical molecule A—B will always be greater than or equal to the arithmetic mean. The difference Δ would never be negative, and will represent the extra ionic resonance energy of the unsymmetrical bond:

$$\Delta = D(A—B) - \tfrac{1}{2}[D(A—A) + D(B—B)]$$

The postulate of the arithmetic mean * is valid for a large number of single bonds, and the values of Δ have been used by Pauling as the basis for formulating an extensive scale of electronegativities of the elements. The Δ values (extra ionic energy) of a number of bonds are shown in Table XII, which gives also the empirical electronegativity differences $(x_A - x_B)$ derived from them by the method described below.

TABLE XII

EXTRA IONIC ENERGY OF BONDS AND ELECTRONEGATIVITY
DIFFERENCES OF ATOMS (PAULING)

Bond	Δ kcal.	$\sqrt{\dfrac{\Delta}{23.06}}$	$x_A - x_B$	Bond	Δ kcal	$\sqrt{\dfrac{\Delta}{23.06}}$	$x_A - x_B$
C—H	6.3	0.52	0.4	Si—F	90.0	1.97	2.2
Si—H	2.1	0.30	0.3	Si—Cl	35.6	1.24	1.2
N—H	22.0	0.98	0.9	Si—Br	25.0	1.04	1.0
P—H	1.8	0.28	0.0	Si—I	11.7	0.71	0.7
As—H	−12.0	——	0.1	Ge—Cl	53.9	1.53	1.2
O—H	41.0	1.33	1.4	N—F	27.0	1.08	1.0
S—H	3.9	0.41	0.4	N—Cl	−0.5	——	0.0
Se—H	−7.5	——	0.3	P—Cl	24.4	1.03	0.9
H—F	64.0	1.67	1.9	P—Br	16.7	0.85	0.7
H—Cl	22.1	0.98	0.9	P—I	7.6	0.58	0.4
H—Br	12.5	0.74	0.7	As—Cl	23.8	1.01	1.0
H—I	1.6	0.26	0.4	As—Br	17.4	0 87	0.8
C—Si	7.0	0.55	0.7	As—I	7.4	0.57	0.5
C—N	9.3	0.64	0.5	O—F	9.4	0.64	0.5
C—O	23.2	1.00	1.0	O—Cl	2.9	0.36	0.5
C—S	6.7	0.54	0.5	S—Cl	5.3	0.48	0.5
C—F	45.9	1.41	1.5	S—Br	2.2	0.31	0.3
C—Cl	8.3	0.61	0.5	Se—Cl	9.1	0.63	0.6
C—Br	1.6	0.26	0.3	Cl—F	15 7	0.82	1.0
C—I	−1.9	——	0.0	Br—Cl	0.7	0.18	0.2
Si—O	50.6	1.48	1.7	I—Cl	4.0	0.42	0.5
Si—S	7.7	0.58	0.7	I—Br	1.7	0.27	0.3

The Electronegativity Scale. The values of Δ are a measure of the ionic character of the covalent bond A—B, and it is observed that Δ increases as the two atoms A and B become more and more unlike in electronegativity; conversely, the Δ values become very small when the two atoms in combination are alike in electronegativity. Thus, in the series H—F, H—Cl, H—Br, H—I, the Δ values are, respectively, 64.0, 22.1, 12.5, and 1.6 kcal./mole, and in the series H—C, H—N, H—O, H—F the values are 6.3, 22.0, 41.0, and 64.0 kcal./mole.

* The geometric mean, $[D(A—A) \times D(B—B)]^{\frac{1}{2}}$, has been shown by Pauling to give somewhat more satisfactory values of Δ, particularly when the symmetrical bond energies differ greatly from each other. The arithmetic and geometric means differ but slightly of course, when the bond energies differ from one another by small amounts.

The property of electronegativity referred to here is the power of an atom *in a molecule* to attract electrons to itself and is closely akin to the intuitive notion of electronegativity as used by organic chemists. This property is different from the electrode potential of the element, or the ionization potential of the atom, or the electron affinity of the atom.

By analysis of the Δ values Pauling was able to assign to the elements electronegativity values which satisfy approximately the relation $\Delta_{AB} = (x_A - x_B)^2$, where x_A and x_B represent the electronegativity values of the atoms A and B. In formulating the electronegativity scale the Δ values are expressed in electron volts (1 e.v. = 23.06 kcal.), since this gives a convenient range, and an additive constant has been chosen to give the first-row elements Li to F the values 1.0 to 4.0.

TABLE XIII

PAULING'S ELECTRONEGATIVITY SCALE OF THE ELEMENTS SHOWING RELATION TO THE PERIODIC TABLE

H
2.1

Li		Be		B		C		N	O	F
1.0		1.5		2.0		2.5		3.0	3.5	4.0

Na		Mg	Al	Si		P	S	Cl		
0.9		1.2	1.5	1.8		2.1	2.5	3.0		

K	Ca		Ge	As	Se	Br				
0.8	1.0		1.7	2.0	2.4	2.8				

Rb	Sr		Sn	Sb	Te	I				
0.8	1.0		1.7	1.9	2.1	2.4				

0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0

x values

This electronegativity scale is particularly useful since it provides definite comparison of atoms from different groups and different horizontal rows of the periodic table. Such information is of value in correlating experimental observations, in the development and refinement of organic chemical theories dealing with the relative tendencies of various atoms or groups to retain the bonding pair of electrons in a reaction, and in other ways. Moreover, by means of Pauling's empirical curve relating the per cent of ionic character for a bond A—B with the electronegativity difference $(x_A - x_B)$, it is possible to obtain a fairly good

estimate of the ionic character of a variety of bonds for which no experimental data are available.

The x values of the electronegativity scale apply to atoms with a formal charge of zero. Pauling has estimated the effect of formal charge on the x values in the following way. The increasing electronegativity for a series of atoms in the same horizontal row, such as C, N, O, F, can be attributed to successive increase in the effective nuclear charge acting on the valence electrons. The successive increments in the x values
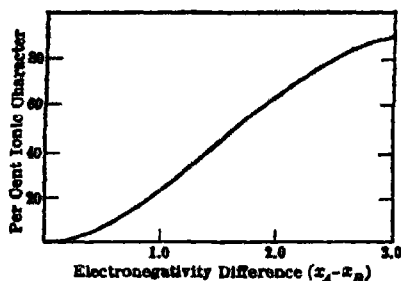
Pauling's empirical curve relating amount of ionic character of a bond A—B to the difference in electronegativity of atoms A and B $(x_A - x_B)$.

in this series (i.e., in going from N of NR_3 to O of OR_2, etc.) correspond to an increase of about 0.6 (in electron charge units) in the effective nuclear charge, this amount being the increase in actual nuclear charge ($+1$) diminished by the screening constant of one valence electron for another (about 0.4). In going from N of NR_3 to $[NR_4]^+$, in which nitrogen has a formal charge of $+1$, the increase in effective nuclear charge will be 0.4 since the actual nuclear charge remains unchanged but the screening effect is diminished by one electron. Thus, a unit positive formal charge will increase the x value for an atom by about $\frac{2}{3}$ of the distance to the next atom to the right in the periodic table; conversely, a unit negative formal charge will decrease the x value by about $\frac{2}{3}$ of the distance to its left neighbor in the periodic table. If an atom forms bonds which are largely ionic in character in addition to the bond under consideration, an estimate of the effect of the actual charge on the x value may be obtained by the same general method used for formal charges.

Bond Polarizabilities. The rupture of a covalent bond in the course of reaction involves factors other than the extent of polarization in the normal state. The presence of permanent residual charges has an orienting influence, but the main consideration is the extent to which the atoms of the bond are capable of undergoing a temporary polarization under the influence of polar centers of the reagent, that is, the polarizability of the bond.

The relative polarizability of covalent bonds depends primarily upon the relative *mobility* of electrons within the systems, and can be deduced from refractivities. Since the refraction of light in the visible region is due to the displacement of electrons and not atomic nuclei, it is possible to assign "constants" to groups of electrons rather than to atoms. Detailed analyses of molecular refractions [23] have led to bond refractivities (symbol P_E), and from these values the bond polarizabilities (symbol α) can be calculated. Since the bond refractivities and bond polarizabilities have a linear relationship ($P_E = \frac{4}{3}\pi N\alpha$), the former may be used directly for the comparison of bond polarizabilities. The polarizabilities of some typical bonds are shown in Table XIV, in which the P_E values given for systems containing unshared electron pairs include the contribution of the unshared pairs.

TABLE XIV

BOND REFRACTIVITIES

Bond	P_E	Bond	P_E	Bond	P_E
H—C	1.70	C—C	1.21	C=C	4.15
H—N	1.8	C—N	1.55	C≡C	6.02
H—O	1.85	C—O	1.43	C=O	3.42
H—F	1.9	C—F	1.6	F⁻	2.4
H—Cl	6.67	C—Cl	6.57	Cl⁻	9.0
H—Br	9.14	C—Br	9 47	Br⁻	12.6
H—I	13.74	C—I	14.50	I⁻	19.0

The effect of electrical charges on electron mobility is shown by comparison of the hydrogen halides and the corresponding halide anions; further illustration is afforded by the series $[OH]^-$, H_2O, and $[H_3O]^+$, where the refractivities are 5.1, 3.76, and 3.0, respectively. The influence of multiple bonds is indicated by comparing C—C with C=C and C≡C; the refractivity of the double bond is 2.075 per electron pair, the triple bond 2.01 per electron pair, and the single bond 1.21. This indicates that the double bond is almost twice as easily polarized as a single bond, but differs very little from a triple bond in its polarizability.[24] The low polarizability of the single bond C—C accounts also for the rapid diminution of inductive effects in a saturated carbon chain and the greater polarizability of C=C for the ability of unsaturated systems to transmit them with much smaller loss.

The refractivities of the bonds C—X follow the same order as the

[23] Fajans and Knoor, *Ber.*, **59**, 256 (1926); see, also, Smyth, "Dielectric Constant and Molecular Structure," Chemical Catalog Co., New York (1931).

[24] Waters, "Physical Aspects of Organic Chemistry," Routledge and Sons, London (1935).

general reactivities of the halides in metathetical reactions involving elimination of the halide anion. It must be recognized, however, that polarizability effects can occur in either direction, and a high polarizability of systems containing an unshared electron pair may be related to the tendency to form additional covalent links by coördination with an acceptor center. The stability or reactivity of the resulting structures involves a number of other factors and cannot be predicted directly from the relative polarizabilities.

CLASSIFICATION OF CHEMICAL REACTIVITIES

Organic molecules may be classified superficially from the standpoint of the electronic configurations of the valence shells of the principal atoms, but the important consideration in dealing with chemical reactions is the behavior of their active centers relative to other systems. The classification of reagents as acids and bases, or as oxidizing and reducing agents, is merely a convenient expression of their activity relative to one another. Within a given category it is possible to place reagents along a scale of relative affinities to express the activity of acids, or oxidizing agents, relative to each other. Obviously, the possession of one dominant characteristic does not necessarily imply the absence of the opposite property; a base will act as such in contact with a reagent that is less basic than itself but can act as an acid in the presence of a reagent more basic than itself.

Acids and Bases. Lewis [1] has given the following broad definition of basicity and acidity: a basic substance is one which has an unshared pair of electrons which may be used to complete the stable group of another atom, and an acidic substance is one which can employ an unshared pair from another molecule in completing the stable group of one of its own atoms.[36] Thus, acetic acid is acidic with reference to hydrocarbons, alcohols, and amines, all of which are relatively stronger electron donors, but it is basic with reference to hydrogen chloride, which is a weaker electron donor. It is possible to arrange molecules and ions in the order of their acidic or basic activity relative to a definite criterion, but it is not to be expected that the identical sequence will be maintained toward all reagents of either type.

Oxidation and Reduction. A consideration of oxidation and reduction shows that the reducing agent donates electrons, or a share in its electrons, to the oxidizing agent. Consequently, oxidation and reduction are analogous to basicity and acidity from the standpoint of the fundamental electronic characteristics of the active centers. Bases

[36] See, also, Luder, *Chem. Rev.*, **27**, 547 (1940).

and reducing agents are electron donors; acids and oxidizing agents are electron acceptors. In any category the reagents may be subdivided into two groups, depending upon whether they act by donating or accepting electrons completely or by a sharing process. For example, an active metal or ion (Na· or Fe^{++}) usually acts as a reducing agent, or as a "base," by donating electrons completely; covalent molecules (SO_2, NH_3, etc.) usually act by donating to an oxidizing agent, or to an acid, a share in previously unshared electron pairs. Sulfur dioxide acts as a reducing agent by virtue of the unshared electron pair in the valence shell of the sulfur atom; it can act also as an oxidizing agent or acid, by virtue of its ability to accept an unshared electron pair from another molecule or ion ([OH]⁻ or NH_3).

$$\begin{array}{ccc} O & & O \\ \uparrow & & \uparrow \\ :S\!=\!O + HO\!-\!X \rightarrow & X\!-\!S\!-\!OH \\ & & \downarrow \\ & & O \end{array}$$

SO_2 as electron donor

$$:S\!=\!O + [OH]^- \rightarrow \left[\begin{array}{c} O \\ \uparrow \\ :S\!-\!OH \\ \downarrow \\ O \end{array}\right]^-$$

SO_2 as electron acceptor
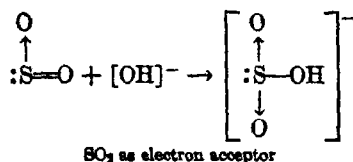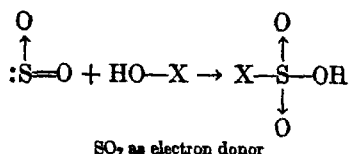
Cationoid and Anionoid Activity. Following Lapworth's proposal that reagents should be classified as anionoid or cationoid according as they resemble active anions or cations in their behavior, Robinson has arranged the active centers of typical reagents into two groups indicating their behavior relative to one another in the course of reactions (Table XV).[*] It must be emphasized that the terms cationoid and anionoid are not intended to imply that the nature of the electro-affinity depends upon the state of polarization of the reactive system. These names refer to the characteristic acceptor or donor activity of reactive cations or anions.

Electrophiles and Nucleophiles. Ingold has designated reagents which donate their electrons to, or share them with, a foreign atomic nucleus as nucleophilic; reagents which acquire electrons, or a share in electrons previously belonging to a foreign molecule or ion, are termed electrophilic. The broad classification of organic molecules and reagents as electron acceptors or electrophiles, and electron donors or nucleophiles, embraces also the narrower classifications (acids and bases, oxidizing and reducing agents). It is evident, therefore, that all the

methods of classifying the electroaffinities of organic molecules and reagents are based upon essentially the same principles and the same electrochemical concept of chemical activity (p. 1840).

<div align="center">

TABLE XV

CLASSIFICATION OF REAGENTS (LAPWORTH-ROBINSON)

Anionoid (Electron-donating)
</div>

Reactive anions: $[NH_2]^-$, $[OH]^-$, $[CN]^-$, $[OR]^-$, $[CH(CO_2Et)_2]^-$, etc.

Molecules containing unshared electron pairs: N of NH_3 and amines; O of H_2O, ethers, aldehydes, ketones; S of mercaptans, sulfides; etc.

Reducing agents: Fe^{++}; metals (as sources of electrons).

Hydrocarbon residues of organometallic compounds: R— of R—MgX; R—C≡C— of acetylides; etc.

Unsaturated carbon of olefins and of aromatic compounds: CH_2=CH_2; C_6H_6.

Neutral atoms and free radicals (common to both classes).

<div align="center">

Cationoid (Electron-accepting)
</div>

Protons and proton sources: acids, etc.

Reactive cations: $[H_3O]^+$, diazonium ions, cations of pseudo bases (e.g., cotarnine).

Metallic atoms with incomplete valence shells, capable of coördination: HgR_2, etc.

Alkyl residues of esters, alkyl halides, and quaternary ammonium compounds: $(CH_3)_2SO_4$, CH_3—X, $[(CH_3)_4N]^+$, etc.

Halogens, ozone, peroxides, and oxidizing agents: CrO_3, Fe^{+++}, MnO_4^-, etc.

Carbon of carbonyl groups (aldehydes, ketones, esters) and nitriles.

Nitrogen of nitroso and nitro compounds, and nitric acid.

Sulfur of SO_2, H_2SO_4, $NaHSO_3$.

Neutral atoms and free radicals (common to both classes).

Formulation of Reaction Mechanisms. It must be recognized that the formal classification of reagents as electrophilic and nucleophilic, or cationoid and anionoid, is based upon considerations of the initial and final states of the reactive centers and is independent of specific hypotheses concerning the intimate mechanism of the reaction process. From the standpoint of reaction mechanisms the intrinsic electroaffinity of an active center, or the overall transition from the initial to the final state, is less significant than the nature of the process through which the electronic transfer is accomplished. The formulation of definite reaction mechanisms depends upon the introduction of various speculative hypotheses designed to correlate the chemical behavior of active centers with their electronic configurations.

A certain confusion arises from the circumstance that the inherent attraction of a reactive center for electrons or for atomic nuclei may operate by a direct or indirect process. There is little doubt, for example, that the driving force of the characteristic reactions of molecular

chlorine arises from an inherent attraction for electrons. Ingold regards chlorine as an electron-accepting reagent; it is considered so in the Lapworth-Robinson classification and placed in the same category with potential alkyl cations, the carbon atom of the carbonyl group, and metallic atoms having an incomplete valence shell. However, in speculating upon the mode of attack of reagents by chlorine it appears that the avidity of chlorine for electrons may be appeased by a circuitous process: one atom can act as a donor in a preliminary attachment to an acceptor center and the other atom then migrates to a donor center of the reagent. The result of the whole process is that both chlorine atoms attain a state in which they have a larger share in the binding pair of electrons than either had in the Cl—Cl link.

$$Cl—Cl + A—B \rightarrow \left[\overset{\delta+}{Cl}—Cl \rightarrow \overset{\delta-}{A}—B \right] \rightarrow Cl—A + Cl—B$$
$$\text{(Substitution)}$$
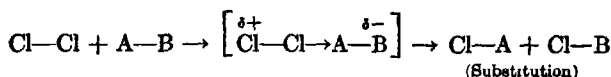
The same reaction can be formulated by the assumption of a preliminary polarization of the chlorine molecule by the electrical field of a molecule of the reactant, or a solvent, or surface on which adsorption has occurred.

$$\overset{\delta-}{Cl}—\overset{\delta+}{Cl} + B—A \rightarrow \left[\overset{\delta-}{Cl}—\overset{\delta+}{Cl} \quad B—A \right] \rightarrow Cl—B + Cl—A$$
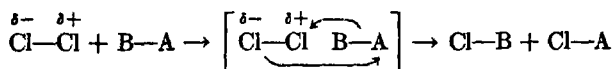
The reaction is then considered to result from the strong electron attraction of the electron-depleted (positively polarized) chlorine atom. The same products would be expected according to either formulation, so that an examination of the final products will not be of assistance in distinguishing between the alternative mechanisms. Indeed, the source of instability is due in both cases to the same active cause, the electron deficiency of the positively polarized chlorine atom.

In the following discussion an effort has been made to incorporate the pertinent features of the important generalizations of Robinson and of Ingold, and to present a composite picture of the contributions of a number of other investigators in the application and extension of electronic theories. Since the formulation of intermediate complexes seems justified on physical and chemical grounds and useful in the interpretation of many effects that are associated with reactivities, particular emphasis has been given to the hypothesis of preliminary coördination complexes. The reactive complexes are intended to represent mobile systems which facilitate the occurrence of effective electron displacements in the course of reaction and must not be confused with stable intermediate compounds that can sometimes be isolated.

CLASSIFICATION OF REACTIONS

The most typical reactions of organic molecules that occur in solutions and at ordinary temperatures may be grouped into three general classes, according to the nature of the reagent: (i) reactions between a covalent molecule and a free atom, especially an alkali metal, or a free radical; (ii) reactions between a covalent molecule and an ionized substance; (iii) reactions between two covalent molecules. It is by no means a simple matter to decide upon the intimate mechanism of all organic reactions, but it may be stated that, in general, only the first class of reactions will involve a symmetrical fission of the binding pair of electrons to yield an electrically neutral fragment, a free radical. These reactions may, therefore, be designated as non-ionic or radical reactions. The second and third classes may be considered to involve an unsymmetrical cleavage in which the electron pair remains intact and is retained by one of the atoms of the link. Reactions of this kind need not be sharply differentiated, and both are frequently called ionic reactions. It is convenient to designate the second class as simple ionic and the third class as pseudo ionic or complex ionic reactions.

The three classes of reactions of covalent molecules usually differ markedly from the ionic reactions of strong electrolytes in that the former take place more slowly. To account for this difference Arrhenius introduced the notion that chemical reaction depends upon the presence of a relatively small number of active molecules, and that a normal or average molecule must be brought to a higher energy level (activated state) before reaction will occur. This conception has been extremely fruitful, and the Arrhenius equation $k = Ze^{-E/RT}$, relating the reaction velocity k with the number of molecular collisions and the energy of activation E, has served as a basis for the general correlation of kinetics of chemical changes. In the activation theory the quantity E represents the amount of energy which a molecule must have in excess of the normal or average energy content, and, since this quantity enters as an exponential term, small variations in E will produce a relatively large effect upon the rate of reaction.

Physical evidence leads to the view that there is a time interval between an effective collision and the occurrence of reaction, and that an "activated complex" intervenes as an intermediate state between the reactants and the products. The driving force of a reaction depends upon energy increments that are associated with polarization and polarizability effects in a covalent structure. In the activated complex relatively small forces are able to bring about a redistribution of the

electronic systems among the atomic nuclei, to give more stable configurations.

Radical Reactions. The interaction of an organic halide and an alkali metal may be taken for consideration of the general mechanism of this class of reactions. The metal transfers an electron to the halide molecule forming a highly unstable organic anion. The transient anionic complex $[R—X+e]^-$ decomposes into X^- and $R—e$. The subsequent transformations will depend upon a number of factors: the intrinsic stability of the free radical, which is a constitutive property determined by internal factors; the nature of the environment; and the relative concentrations of other molecules or atoms with which it can react (external factors). Reaction of the free radical with a second atom of the metal will generate an organometallic compound RM; reaction with another free radical may produce either R—R (Wurtz-Fittig reaction) or R—H and an olefin (disproportionation). It is possible to

$$M\cdot + X—R \rightarrow M^+ + X^- + \cdot R$$
$$R\cdot + M\cdot \rightarrow RM \qquad R\cdot + R\cdot \rightarrow R—R$$
$$R\cdot + R\cdot \rightarrow C_nH_{2n+2} + C_nH_{2n}$$
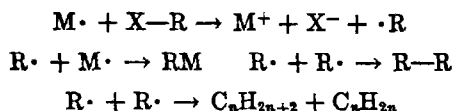
attribute the formation of R—R to the interaction of RM with a second molecule of R—X, and to envisage other mechanisms leading to the same products, but these details need not be considered here (see p. 537).

The relative reactivity of a series of halides (R—F, R—Cl, R—Br, and R—I) toward a given metal should be determined by the relative ability of a collision with the metallic atom to produce an effective electronic displacement in the direction $X\rightarrow—R$. Consequently the polarizability of the C—X bond, and not the residual charge in the link, will be paramount, and the reactivity should decrease from iodide to fluoride. This is verified in the most striking way by ingenious experiments of Polanyi and his collaborators,[26] who have shown that the number of collisions required to produce one effective collision, in the reaction of methyl halides with sodium vapor, is 1.5 for $CH_3—I$, 25 for $CH_3—Br$, 5000 for $CH_3—Cl$, and 10,000,000 for $CH_3—F$. Similar results were obtained with the ethyl and aryl iodides, bromides, and chlorides.

There is some evidence that the attack of sodium on organic halides such as bromobenzene gives rise to transient sodium halyls (p. 539), corresponding to the ketyls obtained from aromatic carbonyl compounds.[27] This suggests that the reactions under ordinary conditions

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[26] Horn, Polanyi, and Style, *Trans. Faraday Soc.*, **30**, 189 (1934).

[27] Morton and Stevens, *J. Am. Chem. Soc.*, **54**, 1919 (1932); see, also, Bachmann and Wiselogle, *ibid.*, **58**, 1943 (1936).

probably occur by the addition of an electron to the halogen atom, through the temporary expansion of its valence shell. In aromatic

$$Na\cdot + Br{-}C_6H_5 \rightarrow Na[\cdot Br{-}C_6H_5]^-$$

Metal halyl

$$Na + Br^- + \cdot C_6H_5$$

$$Na\cdot + R{-}CO{-}C_6H_5 \rightarrow Na^+\left[R{-}\overset{O}{\underset{\cdot}{C}}{-}C_6H_5\right]^- \rightleftarrows Na^+\left[R{-}\overset{O}{C}\right]^-$$

Metal ketyl
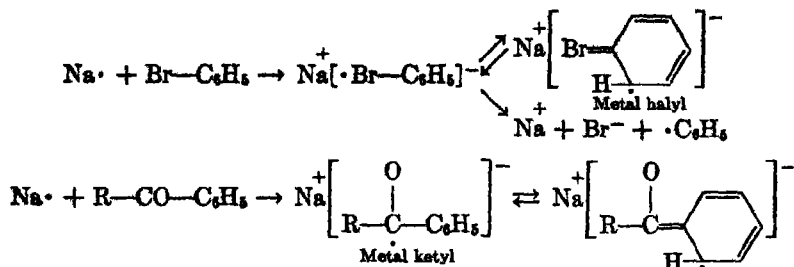
halyls and ketyls the relatively longer life of the intermediate may be attributed to resonance effects. The halyl complex may be regarded as a temporary state which permits internal dynamic effects to become operative.

Simple Ionic Reactions. The metathetical reactions of alkyl halides with hydroxides, alkoxides, and salts of alkali metals are typical of this class. The rate of reaction usually follows the order R—I > R—Br > R—Cl, in accordance with the polarizability of the halogen atoms. The fact that reactions of this kind can occur without the racemization of an asymmetric system attached to the halogen atom, but with optical inversion,[28] indicates that the process may be pictured in the following way:

$$X^- + H{-}\overset{R'}{\underset{R}{C}}{-}Cl \rightarrow X^-\ \overset{R'}{\underset{R}{C}}H{-}\overset{\frown}{Cl} \rightarrow X{-}\overset{R'}{\underset{R}{C}}{-}H + Cl^-$$
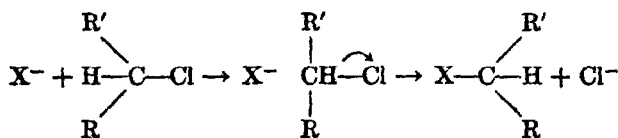
In this case the configuration of the asymmetric center is "turned inside out, like an umbrella in a strong wind." A preliminary separation of *free* alkyl cations in reactions of this kind is quite improbable, since this would lead to racemization and possibly to molecular rearrangement within the alkyl group.

The effect of structural variations of the alkyl group upon the rates of reaction has been studied for many reactions of this type. Although different ionic reagents do not always place groups in an identical sequence, it is generally true that the order of decreasing reactivity is: methyl > primary > secondary > tertiary groups. Aryl and vinyl halides are extremely inert, and allylic or benzylic halides are highly reactive (p. 1053).

Since alkyl groups tend to produce an effect of electron-release relative to hydrogen, it might be expected that the tertiary alkyl halides should be more reactive than the secondary or primary compounds. However, the determining factor here appears to be the mobility of the cationic center, that is, the relative tendency of a collision to produce a sufficient mobility of the respective cationic centers. Since the alkyl groups are electron-releasing groups they reduce the electronic deficit and thereby diminish the probability of an effective collision. Aryl and vinyl groups inhibit reaction through their tendency to favor a mesomeric polarization which increases the covalence of the carbon-halogen bond (see hetero-enoid systems, p. 1909). The interposition of a

$$CH_2\!\!=\!\!CH\!\!-\!\!Cl \rightleftarrows :\overset{-}{C}H_2\!\!-\!\!CH\!\!=\!\!\overset{+}{Cl}$$

Inactivation of halogen in vinyl halides

$$CH_2\!\!=\!\!CH\!\!-\!\!CH_2Cl \rightleftarrows CH_2\!\!=\!\!CH\!\!-\!\!CH_2\!\!-\!\!Cl$$

Activation in allyl and benzyl halides
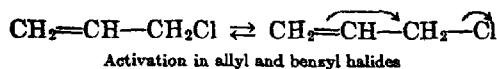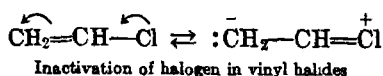
methylene group, as in $CH_2\!=\!CH\!-\!CH_2\!-\!Cl$ or $C_6H_5\!-\!CH_2\!-\!Cl$, has a strong positive effect since the vinyl or aryl system is capable of exerting a strong *dynamic effect* of electron release toward the atom to which it is attached. Mesomeric polarization ($-T$ effect of the halogen atom), which disfavors separation of a halide anion, does not occur in the allyl and benzyl halides owing to the inability of the methylene group to hold an additional electron pair. It will be recalled that dynamic effects are able to facilitate a given type of reaction, but cannot in themselves impede an alternative reaction.

In certain reactions, and especially with elements of high atomic number, the mechanism may involve a temporary expansion of the valence shell beyond an octet (p. 1837). The anomalous hydrolysis of certain halides, particularly iodides, to give hydrocarbons is an example of this phenomenon.

$$R\!-\!I + OH^- \rightarrow [R\!-\!I\!\leftarrow\!OH]^- \rightarrow R\!-\!H + [I\!-\!O]^-$$
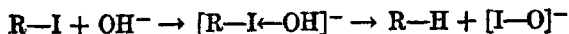
This type of reaction occurs with "positive" halogens, such as $R\!-\!C\!\equiv\!C\!-\!X$, p-amino-aryl halides, certain α-halogenated ketones, etc.

Pseudo-Ionic Reactions. The rates and mechanisms of many reactions between covalent molecules are similar to those observed in simple ionic reactions. There is no fundamental distinction between the reaction of an alkyl halide with an amine, and that with an ionized metallic salt. In this type of reaction, also, optical inversion takes place and not racemization. However, it is frequently observed that

CHELATE RINGS

The term chelate ring denotes a cyclic structure that arises through intramolecular coördination in systems containing a donor and acceptor center, as in salicylaldehyde (I) and the covalent copper salt of glycine (II), or a ring that is formed by intermolecular coördination in systems that are capable of forming two or more coördinate links. The dimers of the carboxylic acids (III), and a variety of metallic complexes derived from ethylenediamine (IV) or anions of dicarboxylic acids, are repre-

sentatives of the intermolecular type. The name "chelate" is derived from the stem *chela*, a pincer-like claw, and was proposed in 1920 by Morgan and Drew.[22] The existence of ring structures in the coordination complexes of ethylenediamine and similar compounds had been known much earlier, but recognition of the importance of the phenomenon of ring closure by coördination and its bearing on chemical problems appears to have been due largely to the work of Morgan and his collaborators.[23] They showed, for example, that mordant dyes are chelate structures and that the ability to dye cloth mordanted with metallic salts is due to structural features of the dye that permit chelation to occur.
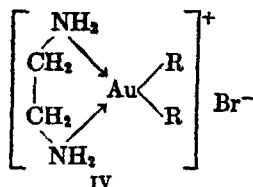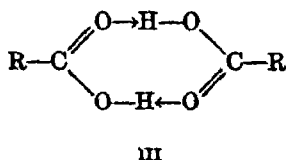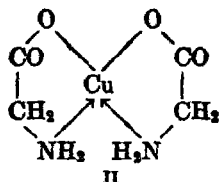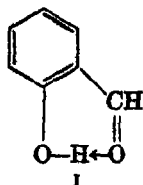
It must be recognized that the process of forming a chelate ring is the same as that leading to the simple open-chain coördination complexes. Indeed, some chelate systems differ very little from the open-chain analogs. However, the chelate rings of special interest in organic chemistry are those in which the cyclization makes possible the occurrence of resonance effects, or serves to alter preëxisting resonance effects.

[22] Morgan and Drew, *J. Chem. Soc.*, **117**, 1457 (1920).
[23] Morgan and Main Smith, *J. Soc. Dyers Colourists*, **41**, 233 (1925).

The existence of chelate structures cannot be doubted; it is supported by experimental evidence derived from stereoisomerism, ionization phenomena, molecular association, solubility behavior, and spectroscopy. Probably the most convincing evidence for the ability of hydrogen to become 2-covalent is afforded by the demonstration that hydrogen can take part in the formation of chelate rings.

In certain *ortho*-substituted phenols the presence of chelate ring structures was postulated by Sidgwick [4] to account for the fact that whenever the substituent has the structure necessary to form a *six-membered* chelate ring (as —CO—H, —CO—R, —CO—OH, —NO₂, etc.) the *ortho* isomer differs markedly from the *meta* and *para* in physical properties and is always less highly associated. The *ortho* isomers are less soluble in water, are more soluble in benzene, and have a lower boiling point.

Although much evidence for the existence of chelate rings involving 2-covalent hydrogen has been adduced from considerations of simple physical properties, the most convincing demonstration has come from infra-red spectroscopy.[34] It has been found that absorption in the region characteristic of the hydroxyl group ($6200\text{--}7500$ cm.$^{-1}$) is absent for a large number of compounds having configurations that would favor formation of a chelate ring containing the hydrogen bond $O\text{---}H \leftarrow O$ or $O\text{---}H \leftarrow N$. The characteristic hydroxyl absorption was retained in related compounds where the constitutions or configurations excluded the formation of such bonds. Thus, the characteristic absorption was absent for phenols containing *ortho* substituents such as —CO—H, —CO—CH₃, —NO₂, and —CO—NH₂, but was present in the *meta* and *para* isomers. Absence of hydroxyl absorption (in 0.1–0.03 molar solutions in carbon tetrachloride) was noted for acetylacetone, benzoylacetone, and dibenzoylmethane, but not in compounds where chelation is excluded on steric grounds.

Chelated enol   Chelation excluded on steric grounds
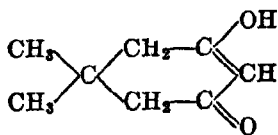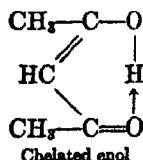
The presence of more than one hydrogen bond within a molecule is shown by the absence of hydroxyl absorption in 1,4- and 1,5-dihydroxyanthraquinone, and 1,4-dihydroxy-5,8-naphthoquinone (naphthazarine). Other interesting examples of the formation of two hydrogen bonds are afforded by 2-naphthol-1-sulfone and 2,2'-dihydroxybenzophenone.

[34] Hilbert, Wulf, Hendricks, and Liddel, *J. Am. Chem. Soc.*, **58**, 548, 1991 (1936).

The former shows also that oxygen atoms held in semi-ionic linkage with sulfur can participate in forming intramolecular hydrogen bonds.[24]
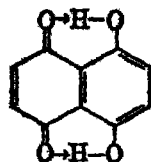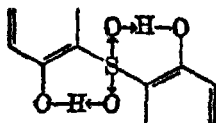
Naphthazarine   2-Naphthol-1-sulfone   2,2'-Dihydroxy-benzophenone
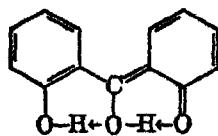
There is evidence that the chelation of hydroxyl groups with *ortho* carbonyl or nitro groups in aromatic systems is accompanied by an increase in color, which may be interpreted as a tendency for the chelation to develop a quinonoid structure within the aromatic ring. The resonating forms of a typical *o*-hydroxyaryl ketone correspond to benzenoid and quinonoid structures, and the color may be considered to be a contribution of the latter. Thus, 2-methoxybenzophenone cannot undergo chelation and is colorless, but 2-hydroxybenzophenone can form a chelate ring and is pale yellow in color. The introduction of an

Benzenoid form   Quinonoid form
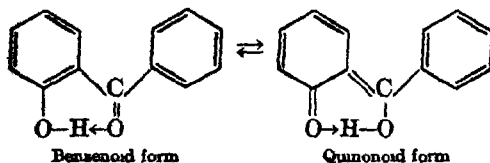
ortho hydroxyl group into the adjacent ring, as in 2,2'-dihydroxybenzophenone, gives a double chelation that is accompanied by a large increase in quinoidation, and the compound is bright yellow.

Metallic derivatives of the enolic forms of β-ketonic esters, β-diketones, and similar tautomeric systems may be ionized salts or covalent chelate rings. The anhydrous form of the sodium derivative of benzoylacetone behaves as a typical ionized salt (I) and is insoluble in hydrocarbons, but it forms a dihydrate which is soluble in toluene and is clearly a covalent molecule containing a chelate system (II). Similar covalent dihydrates are formed by the lithium derivative of benzoylacetone and of methyl salicylate, and by the sodium derivative of aceto-

I   II
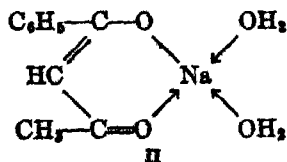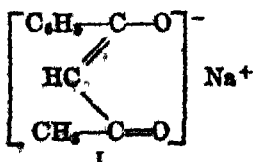
acetic ester. A dichelate system of spirane type is present in the beryllium derivative of acetylacetone (III), and trichelate systems occur in the corresponding aluminum and silicon derivatives.

$$
\begin{array}{ccc}
CH_3-C-O & & O=C-CH_3 \\
\; \diagdown & & \diagup \; \\
HC \quad\quad Be \quad\quad CH \\
\; \diagup & & \diagdown \; \\
CH_3-C=O & & O-C-CH_3 \\
& III &
\end{array}
\qquad
\begin{array}{ccc}
C_6H_5-C=O & & O-C-CO_2H \\
\; \diagup & & \diagdown \; \\
HC \quad\quad Be \quad\quad CH \\
\; \diagdown & & \diagup \; \\
HO_2C-C-O & & O=C-C_6H_5 \\
& IV &
\end{array}
$$
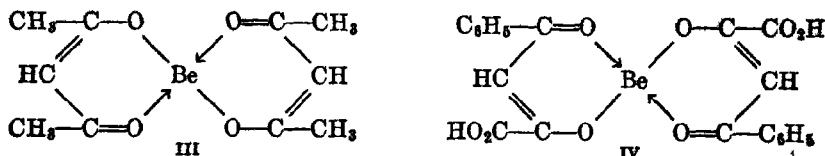
Convincing evidence for the existence of the chelate structures is afforded by the fact that the copper and beryllium derivatives of benzoylpyruvic acid (IV) have been resolved by Mills and Gotts into optically active forms.[15]

Sidgwick[4] has devised a convenient classification of chelate rings into three types, on the basis of the nature of the bonds that are present in the cyclic system.* Rings in which the coördinate link becomes identical with a normal covalent bond as a result of chelation are designated as type A; those containing one or two definite coördinate links are denoted as types B and C, respectively. The ring types are not always sharply differentiated, and frequently, in the liquid state and in solutions, the chelate systems exist in equilibrium with a non-chelate structure. In some cases electromeric (resonance) effects within the system render the classification doubtful.

Type A. These rings result from the chelation of ions and arise through the inability of the central atom to form additional covalent links except by coördination. They are generally quite stable and, in addition to the usual five- and six-membered systems, may contain cycles of four or seven members which are found rarely in other types of chelate rings.

The double carbonates and sulfates of beryllium afford an illustration of four-membered rings of this class (I). Five-membered rings occur in various derivatives of catechol (II); six-membered systems, in the bis-piperidinium salts (III) and in the borosalicylates (IV). All these are relatively stable, and the chelate structures of the last three have been confirmed by resolution into optically active forms.

Certain 1,2-glycols form five-membered chelate ring structures in aqueous solutions of boric acid. The effect of the chelation is to produce a large increase in the acidic strength of the boric acid, owing to

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[15] Mills and Gotts, J. Chem. Soc., 3121 (1926). See, also, Chapter 4, p. 432.

* For an excellent and comprehensive survey of chelate rings see Diehl, Chem. Rev., 21, 39 (1937). In his survey the systems are classified as bidentate, tridentate, etc., on the basis of the number of coördinating groups involved.
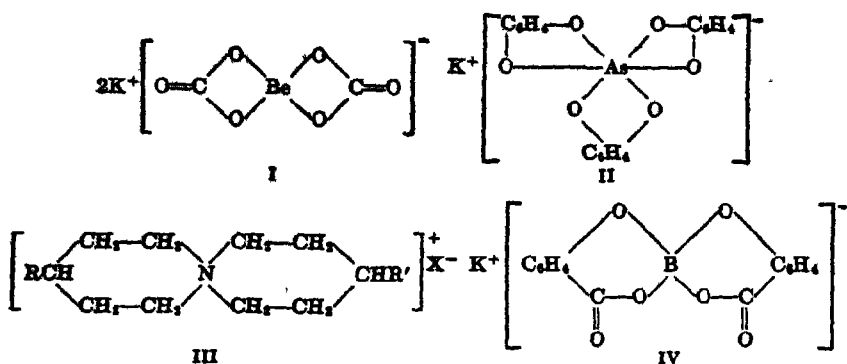
the enhanced stability of the chelate anion. It has been observed, as might be expected, that the spatial configuration of stereoisomeric 1,2-glycols has an influence upon the tendency to form the chelate structures (p. 447). The *cis* form of cyclopentane-1,2-diol or hydrindane-1,2-diol is found to increase the acidic strength of boric acid, but the *trans* form does not; the racemic form of hydrobenzoin has a positive
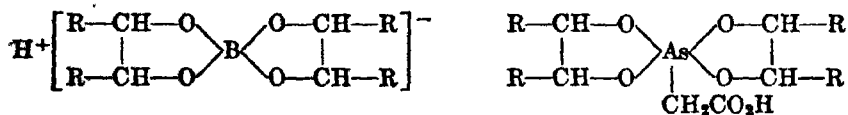
Chelate esters of boric and arsonoacetic acids

effect, but the *meso* form is without effect. The steric and constitutional requirements for the chelation are not perfectly clear, since many aliphatic 1,2-diols (ethylene glycol, 1,2-propylene glycol, and pinacol) have no effect on boric acid and in some cases both pairs of optical enantiomorphs, or *cis-trans* isomers, produce the same effect.[36] The 1,2-glycols also form chelate structures with arsenic acid and with arsonoacetic acid.[37]

Cyclic oxonium compounds, such as the pyrylium and pyroxonium salts (p. 1836), may be regarded as a special case of chelate structures of type A. They are analogous to the bis-piperidinium compounds in that the stability of the ring structure is associated with the presence of a cationic charge produced as the result of a coördination process. With the pyroxonium compounds the ring structure can persist in the absence of an electrical charge, but shows a strong tendency to go over to the open-chain carbonyl compound (under the influence of alkalies).

[36] Böeseken and collaborators, *Rec. trav. chim.*, **39**, 185 (1920); **40**, 525, 553 (1921); **42**, 327, 729 (1922).

[37] Englund, *J. prakt. Chem.*, **122**, 121 (1929).

Type B. In these systems an atom is held in the ring on one side by a normal covalent link and on the other by a coördinate link. The rings are usually less stable than the preceding type and always contain either five or six members. The type B chelate structures most frequently encountered are five-membered rings containing one double bond, and five- or six-membered rings containing two conjugated double bonds. The conjugated rings of this class are probably the most extensive group of chelate structures.

The covalent metallic derivatives of α-ketonic acids, α-amino acids, o-nitrosophenols, mono- and dioximes of o-quinones and 1,2-diketones (dimethylglyoxime, benzil mono- and dioximes), benzoin oxime, 2-pyridyl ketoximes, and 8-hydroxyquinoline are familiar examples of five-membered rings of type B. The oxime complexes have been for-
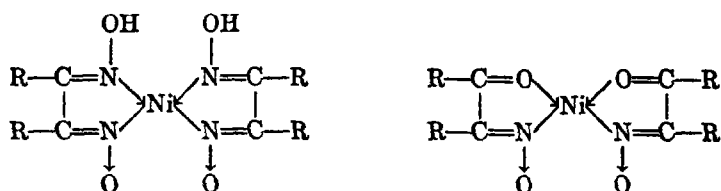
Chelate nickel derivatives of oximes

mulated as six- or seven-membered ring structures, but there is now definite evidence from stereochemistry for the five-membered ring. It is found that the formation of stable metallic complexes occurs only when the configuration is favorable for the structures given above. Thus, complex salts are produced readily from the *anti*-CHOH form of benzoin oxime, the *anti*-2-pyridyl form of 2-pyridyl ketoximes, and the *anti*-form of benzil mono- and dioximes; the *syn*-forms of these oximes do not yield metallic complexes.[88]

Although the saturated compounds (glycols, amino alcohols, diamines, etc.) that could give rise to rings of this type may undergo chelation to some extent, it appears that intermolecular coördination (association) to form open-chain structures occurs more readily. Saturated five-membered rings of Type C are present in metallic complexes formed from 1,2-glycols, 1,2-amino alcohols, and 1,2-diamines, but there is little evidence to support the view that these substances form Type B chelate rings involving 2-covalent hydrogen. Crystal structure analysis of a number of compounds indicates that the valence angle of 2-covalent hydrogen is 180° and that a distance of about 2.6 Ångström units for the O—H ← O or O—H ← N systems is favorable. The chelate

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[88] Meisenheimer and Theilacker, in Freudenberg's "Stereochemie," Deuticke, Leipzig and Vienna (1933), pp. 1039 ff.

forms of saturated 1,2-diols (I) and related types, and of analogous aromatic compounds (II) where a double bond is present, would involve

Chelation restricted by ring strain
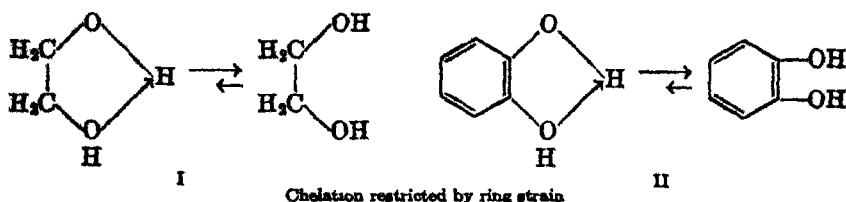
large deviations from the normal valence angles and would therefore be relatively unstable. Evidence from infra-red spectroscopy shows that the characteristic hydroxyl absorption is present in compounds such as catechol and benzoin, but other physical properties of catechol and o-aminophenols suggest that chelation may occur to some extent.

Saturated 1,3-glycols and β-hydroxy carbonyl compounds fulfill the necessary geometrical considerations for the formation of six-membered rings containing hydrogen bonds. However, it is evident that these conditions alone do not suffice since typical examples of such compounds (β-hydroxybutyraldehyde, and esters of tartaric acid) show absorption in the region characteristic of the hydroxyl group. In these cases the diminished tendency to form intramolecular hydrogen bonds may be attributed to the freedom of rotation about the single bonds and the absence of stabilizing resonance effects that can occur in the corresponding unsaturated types (enol forms of 1,3-diketones, etc.).

Six-membered rings containing two conjugated double bonds and one coördinate link are very frequently encountered and are probably the most important chelate rings in organic chemistry. Owing to the favorable steric relations and the intervention of resonance effects, these rings are relatively stable. Either hydrogen (becoming 2-covalent) or a metallic atom acts as the acceptor center, and nitrogen or oxygen acts as the donor atom. Typical examples of chelation through hydrogen are the o-substituted phenols (p. 1868) and enolic forms of β-diketones, β-ketonic esters, and other tautomeric systems. Many of the metallic derivatives of these substances form unusually stable covalent chelate structures; the beryllium, aluminum, copper, and certain other metallic derivatives of acetylacetone can be distilled without appreciable decomposition.

Boron and silicon give stable chelate cations with acetylacetone and similar compounds. The alkali metal derivatives of acetylacetone, and of enolic systems in general, are usually open-chain ionized salts and show little tendency to form chelate structures (lithium >

sodium > potassium). However, in these compounds the stability of the chelate form may be increased by further coördination with a molecule of the free diketone or with a solvent, as in the covalent dihydrates already cited (formula II, p. 1870).

Chelate cation   Chelate lithium enolate
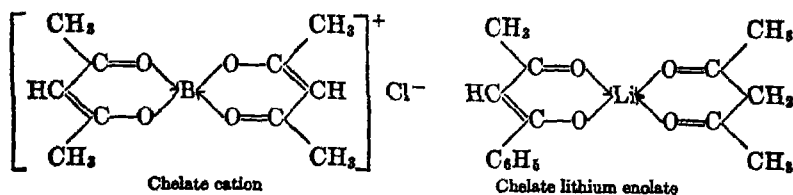
A number of six-membered rings of this class contain oxygen and nitrogen, and occasionally sulfur. Several of the typical chelate systems containing nitrogen are shown in the general formulas I–VI, where M may be hydrogen or a metal and one of the double bonds is frequently part of an aromatic structure. Formulas I and II represent
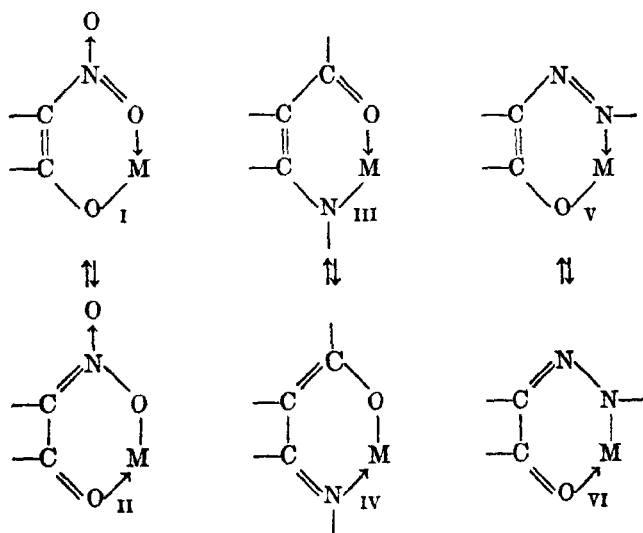
Resonating forms of six-membered conjugated rings

o-nitrophenols; similar types appear to be formed also by o-hydroxysulfones. Formulas III and IV include β-aminocrotonic esters, indigo, anils of o-hydroxy aromatic aldehydes, and o-amino aromatic carbonyl compounds. o-Hydroxyazo compounds, hydrazones of α-ketonic esters, and monohydrazones of 1,2-diketones are examples of formulas V and VI.

Type C. Rings containing two coördinate links are generally the least stable of the three types owing to the fact that relatively stable

molecules (or ions) are formed when the ring is broken. The most common examples of this type are encountered in complexes involving a powerful coördination center such as cobalt, nickel, iridium, or platinum. Five- and six-membered rings are found in the complex ammines containing ethylenediamine and trimethylenediamine, 1,2,3-triaminopropane, 2,2'-bipyridyl, 2-aminomethylquinolines, etc.

Evidence that the saturated five-membered rings are formed more readily than similar six-membered rings is afforded by the mode of chelation in the compound of platinum chloride and 1,2,3-triaminopropane.
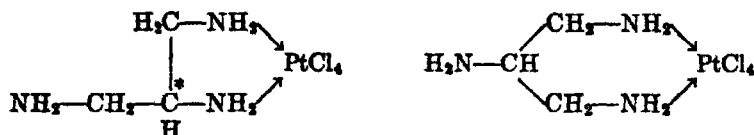
The isomeric five- and six-membered cycles differ in that the former has an asymmetric carbon atom and the latter has not. The product was resolved by Mann and Pope [39] and consequently must have a five-membered ring structure.
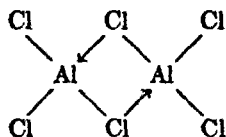
A good deal of evidence supports the view that the dimeric covalent halides of the trivalent metals, such as aluminum and ferric chlorides, are four-membered rings of this type. Since aluminum and iron are able to assume a plane configuration for four covalences the strain in these four-membered chelate structures is reduced. Eight-membered rings containing two coördinate bonds and two double bonds occur in the dimers of the carboxylic acids. Owing to the circumstance that the valence angle of 2-covalent hydrogen is 180°, the symmetrical eight-membered ring (formula III, p. 1868) involves no greater strain than a six-membered ring.[40] The chelate structure accounts for the observed low dipole moment and the fact that polymerization does not proceed beyond double molecules.

Lewis and Schutz [41] have made the interesting observation that replacement of the acidic hydrogen of acetic acid by its heavier isotope, deuterium, brings about a decrease of acidic strength and a slight increase in vapor pressure. Both these changes are attributed to a

[39] Mann and Pope, *Nature*, **119**, 351 (1927); Mann, *J. Chem. Soc.*, 1224 (1927).

[40] Sidgwick, *Ann. Repts. Chem. Soc. (London)*, **30**, 115 (1933).

[41] Lewis and Schutz, *J. Am. Chem. Soc.*, **56**, 493, 1002 (1934).

greater stability of 2-covalent deuterium, which results in an increase in the extent of association to form dimeric molecules.

Polydentate Chelate Rings. Organic molecules containing two coördination centers frequently give rise to di- and tricyclic systems of spirane type, as indicated in a number of structures previously cited (e.g., formulas III, IV, p. 1872). When three or more coördination centers are present, condensed chelate structures may be produced, and these have been designated as tri- and quadridentate systems. A tridentate structure, analogous to the condensed rings of naphthalene, is present in the metallic complexes of diethylene triamine (I), and a quadridentate system (II) in the complexes from bis-acetoacetonyl ethylenediamine.[42]
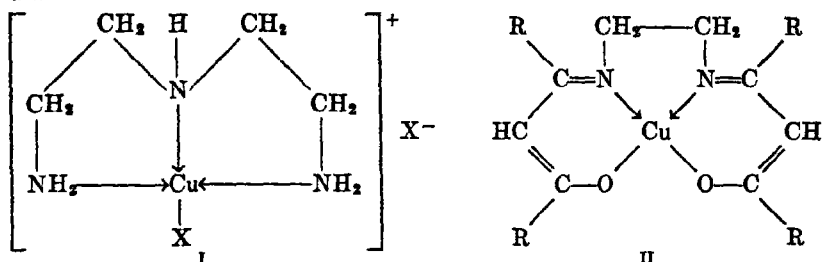
A particularly interesting quadridentate structure is produced from the phthalocyanines; these metallic complexes are obtained readily by the action of iron or magnesium oxide upon o-cyanobenzamide and derive their name from their deep blue color.[43] There is a close structural resemblance between the phthalocyanines and the porphyrins, which form the basis of many important natural pigments (hemoglobin, chlorophyll) and have been shown to contain the "porphin" ring system.
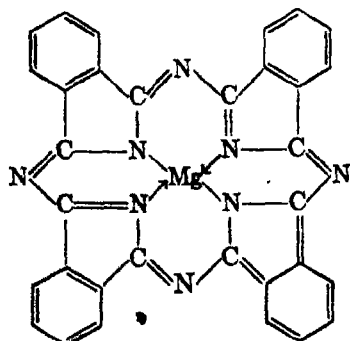
Magnesium derivative of phthalocyanine
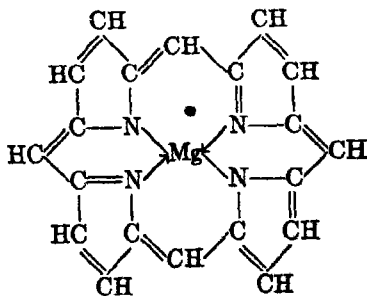
Magnesium derivative of porphin type

[42] Morgan and Main Smith, *J. Chem. Soc.*, 912 (1926).

[43] Linstead and collaborators, *ibid.*, 1016, 1031, 1033 (1934); *Ann. Repts. Chem. Soc. (London)*, **32**, 361 (1935).

The synthetic phthalocyanines bear a close analogy to the natural porphin structure, but are different in two features: each of the four pyrrole units of the phthalocyanines bears a condensed benzene ring in the 3,4-positions, and the units are connected by nitrogen instead of CH groups. These differences do not influence the molecular configuration or stability very seriously, and there are strong resemblances between them. Both are stable to alkalies, less so to acids; both are highly colored and form metallic complexes of similar stability. Thus, the magnesium derivative of a porphin type (phytochlorin, phytorhodin) or a phthalocyanine is intermediate in stability between the potassium salt, which is de-metalated in dilute alcohol, and the very stable copper derivative.

Orientation Effects of Chelation. A definite influence of the effect of conjugation in a chelate structure upon the mobility of the double bonds in an aromatic system has been demonstrated by Baker and his collaborators.[44] Physical properties indicate that 2,4-diacetyl-resorcinol (I) is fully chelated and that this has an effect of fixing the
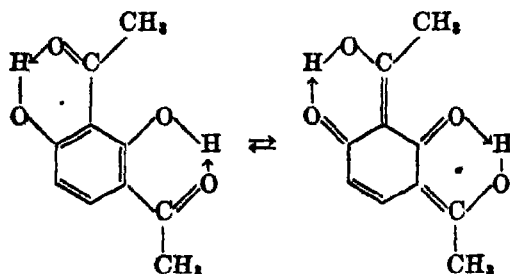
I. 2,4-Diacetylresorcinol (phenanthrene type);
m.p. 89°, b.p. 188°/10 mm.; volatile with steam.
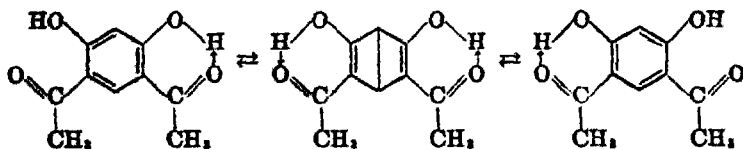
II; 4,6-Diacetylresorcinol (anthracene type);
m.p. 182°, b.p. 188°/10 mm.; non-volatile with steam.

positions of the double bonds of the aromatic structure (p. 140). In the isomeric 4,6-diacetylresorcinol (II), the chelation appears to be less complete since the effects of the chelation upon the double bonds in the aromatic system would oppose each other, and effective participation of the aromatic system would require the production of a *para*-bridged, quinonoid structure. The two systems are analogous to phenanthrene

44 Baker and collaborators, *J. Chem. Soc.*, 1684 (1934); 628 (1935); 274, 346 (1936).

and anthracene, respectively, and there should be little or no fixation of the aromatic double bonds in the 4,6-isomer.

On the basis of the chelation theory Baker predicted that in the Fries reaction 4-acetoxy-2-hydroxyacetophenone (I) should give 2,4-diacetyl-resorcinol rather than the 4,6-isomer. The reaction was found to give about 60 per cent of the predicted product and 40 per cent of the 4,6-isomer. This result indicates a marked orientation effect since the methyl ether of 4-acetoxy-2-hydroxyacetophenone (II), which cannot be chelated, gives almost entirely the 4,6-derivative.

Similar effects were observed in the rearrangement of 4-allyloxy-2-hydroxyacetophenone. The principal product was the 3-allyl derivative, but if the original compound was methylated before rearrangement, the allyl group entered the 5-position.

$$\left(\begin{array}{c}R = \text{acetyl} \\ \text{or allyl}\end{array}\right)$$

I
"Oriented" rearrangement

II
"Normal" rearrangement

The influence of chelation upon the alkaline cleavage of N-acylated benzoin oximes has been suggested by Blatt [45] to account for marked differences in the behavior of the α- and β-forms. The facile cleavage into benzonitrile and benzaldehyde occurs only with the α-(anti-CHOH)-forms, and the β-forms are merely deacylated by alkalies. Examination of isomeric N-acetylated o-hydroxybenzophenone oximes reveals a similar effect; only the anti-hydroxyaryl forms undergo smooth rearrangement to benzoxazoles and the syn-forms are simply deacylated.

Chelation in Chemical Reactions. It has been stated that the powerful catalytic effects of certain metals and salts may be attributed to the formation of unstable coördination complexes, and in certain cases the observed course of reaction suggests that a transitory chelation takes place in the unstable complex (p. 1867). This hypothesis affords a new point of view for the interpretation and correlation of reactions that are not adequately elucidated by the conventional mechanisms. Specific applications of the hypothesis of transient chelation may be illustrated by a consideration of certain "abnormal" reactions of Grignard reagents (pp. 516 and 1881).

. Studies of the behavior of benzylmagnesium chloride toward a variety of reactants have shown that certain carbonyl compounds (formaldehyde, ethyl formate, acid chlorides, and anhydrides) give rise to *o*-tolyl derivatives, but a number of others (carbon dioxide, ketones, and typical esters) produce only the expected benzyl compounds.[46] The experimental evidence shows clearly that the reactant itself plays an important role; the assumption of dynamic isomerism between a normal and *o*-quinonoid form of the Grignard reagent, or rearrangement of a *free* benzyl anion in the course of reaction, does not give a satisfactory account of the observed results. There is also definite evidence against either of these assumptions.

On the basis of the chelation theory,[31] the normal and abnormal reactions are regarded as two possible courses of transposition within the initial coördination complex which is formed as the first step in all Grignard reactions. A carbonyl compound A—CO—B combines with the Grignard reagent, by means of an unshared electron pair of the carbonyl oxygen, to give the initial complex I. The coördination process $C{=}O \rightarrow Mg$ tends to favor electron withdrawal by the benzyl group in the link Mg—$CH_2C_6H_5$, and to promote in the carbonyl group an electromeric displacement $C{=}O$ which would leave the carbon atom with a sextet of electrons (marked by an asterisk, formula II). The normal Grignard reaction occurs by a direct α,γ-shift of the benzyl group with its binding electrons, and without internal rearrangement, to the deficient carbon of the carbonyl system. The octet of the magnesium atom is completed by the usual coördination with ether, and the stable normal product results (III).
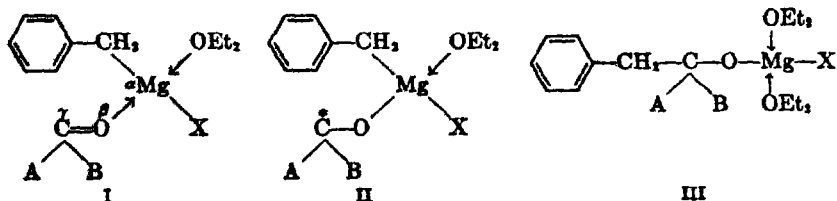
The abnormal reaction arises as a result of the ability of the allylic system in the Grignard reagent to forestall the normal reaction by furnishing the mobile electron pair of the *ortho*-double bond to the deficient carbon atom. The ephemeral chelate ring is broken by rupture of the magnesium-carbon linkage (and coördination of the magnesium with ether) to give the product V.

⁴⁶ Gilman and Kirby, *ibid.*, **54**, 345 (1932); Austin and Johnson, *ibid.*, **54**, 647 (1932)
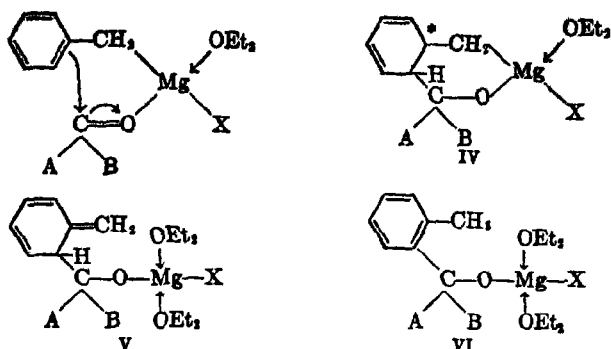
In aliphatic allylic systems the reaction may go no further, but with the aryl compounds a proton shifts to the side chain and completes the conversion of the benzyl group into an o-tolyl group. The tendency of a series of carbonyl compounds to bring about the abnormal reaction is clearly influenced by the nature of the atoms A and B, and parallels the reactivity in typical carbonyl reactions: the most active carbonyl compounds favor the abnormal reaction.

The allylic rearrangements observed by Prévost [47] in the reaction of R—CH=CH—CH$_2$Br and R'—MgX, to give R—CHR'—CH=CH$_2$ and the normal product R—CH=CH—CH$_2$—R', may be explained by a mechanism analogous to that given above. In these cases the allylic group of the reactant is responsible for the abnormal reaction; furthermore, the process is arrested at the stage corresponding to structure V. Obviously the double bond is less mobile here than in an o-quinonoid structure. Other Grignard reactions that appear to involve an ephemeral cyclization are the 1,4-addition reactions of α,β-unsaturated ketones and esters,[48] and o-phenylations of benzophenone anil [49] and highly
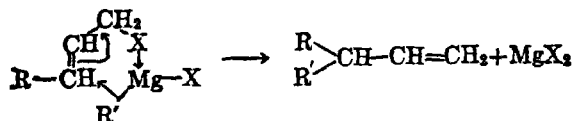
Abnormal reaction of Grignard reagents and allylic halides

substituted α, β-unsaturated ketones [50] by forced reaction with phenylmagnesium bromide.

In the initial complex derived from an α-β-unsaturated carbonyl

[47] Prévost, *Ann. chim.*, [10] 10, 121 (1928); Prévost and Daujat, *Bull. soc. chim.*, [4] 47, 588 (1930); see, also, Carothers and Berchet, *J. Am. Chem. Soc.*, 55, 2813 (1933).

[48] Kohler, *Am. Chem. J.*, 38, 511 (1907), and later papers; see also, pp. 506 and 672.

[49] Gilman, Kirby, and Kinney, *J. Am. Chem. Soc.*, 51, 2252 (1929).

[50] Kohler and Nygaard, *ibid.*, 52, 4128 (1930).

compound (I) two courses of reaction are possible: (i) the "normal" α,γ-shift of the Grignard group R to give 1,2-addition; (ii) shift of the R group (ephemeral cyclization) to the β-carbon of the carbonyl system,
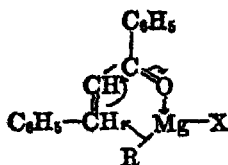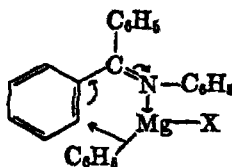
I. 1,4-Addition   II. *ortho*-Phenylation

in concurrence with an electron drift toward the carbonyl group. In this type of reaction it is observed that the most reactive carbonyl systems (aldehydes) (p. 1921) favor 1,2-addition, and less reactive types ($-CO-C_6H_5$, $-CO-OR$) 1,4-addition. *o*-Phenylation (II) of an aryl group attached to the carbonyl system occurs only when steric factors interfere with the 1,2- or 1,4-addition.

The chelation hypothesis is of rather general application and is not restricted to abnormal reactions. The O- and C-alkylation of metallic enolates represents alternative courses of reaction that are analogous to the examples given above: α,γ-shift leads to O-ethers, and the cyclic mechanism to C-alkylation. The Kolbe synethesis and the Reimer-Tiemann reaction may also be formulated by a cyclic mechanism.[21] It may be pointed out, however, that the mere circumstance that a plausible cyclic mechanism can be written for a reaction does not indicate *per se* that the reaction can take place only by a cyclization process. Thus, the rearrangement of the allyl phenyl ethers [51] may occur by
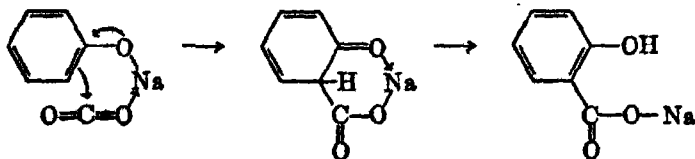
Chelate formulation of the Kolbe synthesis

an intramolecular (cyclization) process, but it may also take place by an intermolecular alkylation.[52] The chelation hypothesis of transient cyclization has this advantage: it offers a definite basis for predicting or correlating the influence of structural factors, or variations in experimental conditions (nature of the medium, etc.), upon the course of a

[51] Ingold, *Ann. Repts. Chem. Soc. (London)*, **23**, 134 (1926); see, also, Tarbell, *Chem. Rev.*, **27**, 495 (1940).

[52] Smith, *J. Am. Chem. Soc.*, **56**, 717 (1934)

given reaction. In a number of instances the observed effects are in good agreement with those anticipated from theoretical considerations.

Intramolecular rearrangement (cyclic)

Intermolecular alkyation
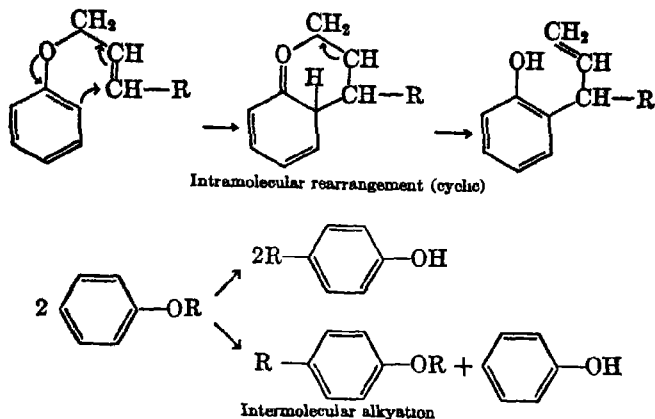
In the rearrangement of benzyl and α-furfuryl phenyl ethers the participation of the allylic double bond in the cyclization (intramolecular) mechanism is diminished by virtue of conjugation in the ring system of benzene or furan, and consequently the intermolecular mechanism is favored. In both these cases the "rearrangement" is observed to occur preferentially in the *para*-position, and a certain amount of the *para*-alkylated ether and free phenol can be isolated from the reaction mixture.

ELECTRONIC CHARACTERISTICS OF TYPICAL BONDS

Unsymmetrical Single Bonds. If the single links of carbon with other elements are regarded from the standpoint of the electronic configuration of the valence shell of the hetero atom in the compound, they fall into three broad classes: (1) links in which the hetero atom has an incomplete valence shell and would require one, two, or three additional electron pairs to form an octet; (2) those in which the hetero atom has a completely shared octet (doublet, in the case of the C—H link) but is capable of increasing its covalence and acquiring additional shared electron pairs by coördination; (3) those in which the hetero atom has an octet containing one, two, or three unshared electron pairs. The first category embraces atoms in Groups I, II, and III of the periodic table; the second, hetero atoms in the higher periods of Group IV; and the third includes hetero atoms in Groups V, VI, and VII. The C—H bond and unsymmetrically substituted C—C bonds may be regarded as special cases in the second class, but they merit individual consider-

ation. Typical examples illustrating the general classification are shown below.

TABLE XVI

CLASSIFICATION OF LINKS BETWEEN CARBON AND HETERO ATOMS

Class 1	Class 2	Class 3
Li—C_2H_5	$(C_2H_5)_3Si$—C_2H_5	$(C_2H_5)_2N$—C_2H_5
C_2H_5—Be—C_2H_5	$(C_2H_5)_3Ge$—C_2H_5	C_2H_5—O—C_2H_5
C_2H_5—Zn—C_2H_5	$(C_2H_5)_3Sn$—C_2H_5	F—C_2H_5
$(C_2H_5)_3B$—C_2H_5	$(C_2H_5)_3Pb$—C_2H_5	I—C_2H_5
etc.	*etc.*	*etc.*

Class 1 (Groups I, II, III). In links of the first class, owing to the lower effective nuclear charge of the hetero atom relative to carbon, a permanent inductive displacement (I_s) will occur toward the carbon atom. These hetero atoms will be considered to exert a negative inductive effect upon the carbon atom, and the latter a positive effect on the hetero atom. When these links are ruptured in the course of reaction the inductive effects will facilitate the separation of the organic group *with* the binding pair of electrons, but the mechanism of reaction involves a consideration of the contribution of coördination processes and of polarizability effects.

A rough estimate of the amount of ionic character in the bonds of carbon with various metallic elements can be obtained by using Pauling's empirical curve relating the amount of ionic character to the difference in electronegativities of the atoms $(x_A - x_B)$. The amount of ionic char-

TABLE XVII

Link	$(x_C - x_M)$	Ionic Character, per cent	Link	$(x_C - x_M)$	Ionic Character, per cent
Cs—C	1.8	55	Be—C	1 0	22
K—C	1.7	50	Al—C	1.0	22
Na—C	1.6	47	Sn—C	0.8	15
Li—C	1.5	43	Ge—C	0.8	15
Ca—C	1.5	43	Sb—C	0 7	11
Mg—C	1.3	34	B—C	0.5	6

acter estimated in this way merely indicates the general trends and does not take into account the influence of substituents on the atoms involved in the link.

In ethylsodium, and the alkali alkyls in general (p. 525), the normal state of the molecule is essentially ▓▓▓▓▓▓▓ent. The decomposition of ethylsodium, ▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓ide and

ethylene may be attributed to the instability of the *free* ethyl anion.[13]

$$Na^+\left[:\overset{\frown}{CH_2-CH_3}\right]^- \rightarrow CH_2{=}CH_2 + Na^+[:H]^-$$

The alkali alkyls are valuable diagnostic reagents for proton mobility,[14] and their high proton affinity, or nucleophilic activity in a broader sense, is likewise to be associated with the intervention of alkyl anions. The separation of free hydrocarbon anions cannot be doubted in the case of the *α*-phenylated alkyls; benzylsodium and its analogs are highly colored substances, and their solutions in appropriate media are electrical conductors.

The reactions of the alkali metal compounds are not usually typical of the behavior of the links of other hetero atoms in this class, and in general the assumption of *free* hydrocarbon anions as intermediates is dubious and unwarranted by the experimental facts. Reactions of the *typical* links appear to involve two steps: the formation of a primary unstable coördination complex in which the hetero atom acts as an acceptor, and a subsequent migration (usually an *α,γ*-shift) of the nascent hydrocarbon anion *within* the complex. There can be little doubt that the initial step in the typical reactions of the Grignard reagents is a coördination process in which the magnesium acts as an acceptor and the reactant furnishes an active donor center; much experimental evidence supports the view that nearly all the hetero atoms in this class act in a similar way.

$$CH_3{-}Zn{-}CH_3 + R{-}I \longrightarrow CH_3{-}Zn{-}\cdots{-}I{-}R \longrightarrow CH_3{-}Zn{-}I + R{-}CH_3$$
$$\underset{\underset{\alpha,\gamma\text{-shift}}{CH_3}}{}$$

$$CH_3{-}Zn{-}CH_3 + H{-}O{-}R \longrightarrow CH_3{-}Zn{-}\cdots{-}O{-}R \longrightarrow CH_3{-}Zn{-}OR + H{-}CH_3$$
$$\underset{CH_3 \rightarrow H}{}$$

$$CH_3{-}Zn{-}CH_3 + 2R{-}O{-}R \longrightarrow \begin{matrix}CH_3 & OR_2 \\ & Zn & \\ CH_3 & OR_2\end{matrix} \quad \text{(stable complex)}$$

In the presence of donor reactants the alkali metal compounds may behave in the same fashion, and in solutions in relatively non-reactive donor solvents (aliphatic ethers and tertiary amines) they may exist as unionized solvated complexes in equilibrium with hydrocarbon anions and solvated cations.

The distinctive reactions of the alkali alkyls appear to be associated with a facile inductomeric polarization, as a result of which they are

[13] Carothers and Coffmann, *ibid.*, **51**, 568 (1929).
[14] Conant and Wheland, *ibid.*, **54**, 1212 (1932).

capable of transferring an alkyl anion to an acceptor reactant without the intervention of a donor center of the reactant or of a donor solvent. For instance, ethylcesium, -rubidium, and -sodium are capable of converting diethylzinc into the triethylzinc anion, from which the volatile diethylzinc (b.p. 118°) cannot be removed by heating. The alkali alkyls react also with the H—C link of benzene, the aliphatic H—C link of toluene, and with ethylenic double bonds. None of these reactions appears to occur with Grignard reagents [45] or with other metal alkyls of Groups I, II, and III.

$$Na—C_2H_5 + Zn(C_2H_5)_2 \rightarrow Na^+\left[\begin{array}{c} C_2H_5 \\ | \\ C_2H_5—Zn—C_2H_5 \end{array}\right]^-$$
$$\text{m.p. 27°}$$

$$Na—C_2H_5 + H—CH_2—C_6H_5 \rightarrow Na^+[CH_2—C_6H_5]^- + C_2H_6$$
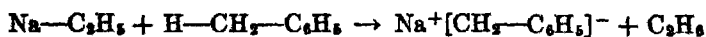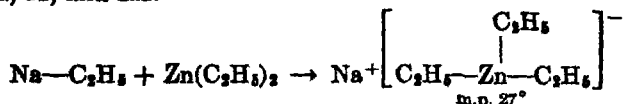
It is of interest to compare the behavior of R—Na, R—Mg—X (or R_2Mg), and R_2AlX (or R_3Al) toward the same reactant. With acetone, the first reacts mainly as an enolizing agent, the Grignard reagent gives an addition product that yields a tertiary alcohol upon hydrolysis, and the organoaluminum compound produces mainly mesityl oxide and higher condensation products. With certain other reactants the organoalkali compounds and the corresponding Grignard reagents yield identical products and differ merely in their rates of reaction (p. 524).[46] Explanations of the observed differences in behavior of organometallic compounds can be inferred from a consideration of relative polarization and polarizability effects within the reacting molecules (internal factors) and the influence of the environment (external factors).[*] The position of equilibrium and the mobility (rate of change) within a system are independent variables; the nature of the products of a reaction will depend upon the relative importance of the contributions of the two factors and their influence upon competitive reactions (p. 1034).

In links of the first class the magnitude of the inductive polarization effects $(- I_s)$ and the tolerance of the hetero atom for a positive charge (polarizability) change in the same way, and vary in a regular manner with the position of the hetero atom in the periodic table. Both quantities diminish as the atom moves to the right in the first two periods (Li > Be > B and Na > Mg > Al), and increase in passing from the first to the second period within each group. Polarization and polarizability increase in passing into the A-subgroup toward the elements of

[45] Gilman and Kirby, *ibid.*, **55**, 1265 (1933).

[*] Internal factors involve the influence of substituents and their mutual interaction, atomic dimensions, and steric effects; external factors take into account the electronic characteristics of the medium and catalysts (if any), and the effect of temperature, concentration, photochemical excitation, etc.

higher atomic number (Li < Na < K < Rb) but decrease in going into the B-subgroups (Mg > Zn > Cd > Hg). The factors associated with the direction of these changes are the effective nuclear charge and the size of the atom (effective atomic and ionic radii)* and its electronic configuration. The marked differences between elements in the A and B subdivisions of Groups I, II, and III involve the relatively smaller effective radii of the elements of the B-subgroups (p. 1888) and the fact that their atomic kernels do not possess an electronic configuration of inert gas type.

The relative acceptor activity of the hetero atoms in Groups I, II, and III may be approached in a roughly qualitative way from the aspects of residual charges in the link, the effective nuclear charges and atomic radii, and the nature of the electronic configurations. The tendency of an atom to ionize and its ability to act as an acceptor (or a donor) are independent properties and are complementary in nature. Both may be seen to proceed from the operation of two fundamental principles: the tendency of an atom to approach the stable electronic configuration of an inert gas, and to achieve a maximum electronic neutralization of its nuclear charge (minimum residual atomic charge).

As the permanent polarization in a covalent link becomes larger the dynamic increment (activation) required for the withdrawal of the binding electrons by the incipient anion becomes smaller, but at the same time there is an increase in the electrostatic attraction between the atoms and an increase in their tendency to form additional links by coördination (subject to the maximum covalence rule, p. 1829). From considerations based upon the optical properties of inorganic salts, Fajans [44] has shown that ions are not rigid structures, and has related the process of ionization to the mutual deforming power of the potential ions (polarization effects) and their susceptibility to deformation (deformability, polarizability).

In inorganic salts the deformation is essentially that of the anion under the influence of cation as a deforming agent; but in the case of a small anion and a large cation (as in potassium fluoride) the effect of the anion may predominate. Fajans observed that the amount of deformation in inorganic salts is greater: (i) the larger the ionic charge; (ii) the smaller the cation; (iii) the larger the anion; (iv) for cations that do not possess an inert gas configuration. These generalizations are in agree-

* The term effective radius is used to indicate the contribution which the atom may be regarded as making to the distance between the two atomic nuclei in the link. The effective radius of an atom increases in passing from an electrically neutral state to that of an anion, and diminishes in becoming a cation.

[44] Fajans, "Radioelements and Isotopes: Chemical Forces and Optical Properties of Substances," McGraw-Hill Book Co., New York (1931).

ment with those predicated from theoretical considerations, and the
known behavior of a large number of inorganic compounds supports the
inference that the tendency of a covalent molecule to ionize is restricted
by the amount of deformation of the potential ions.[4, 5] The approx-
imate radii of some of the typical univalent ions, calculated by Pauling [57]
are shown in Table XVIII.

TABLE XVIII

APPROXIMATE UNIVALENT CRYSTAL RADII OF IONS (PAULING)
(in Ångström units)

Univalent Cations

Li	Be	B	C
(0.60)	(0.44)	(0.35)	(0.29)
Na	Mg	Al	Si
(0.95)	(0.82)	(0.72)	(0.65)

A-Subgroups		B-Subgroups			
K	Ca	Cu	Zn	Ga	Ge
(1.33)	(1.18)	(0.96)	(0.88)	(0.81)	(0.76)
Rb	Sr	Ag	Cd	In	Sn
(1.48)	(1.32)	(1.26)	(1.14)	(1.04)	(0.96)
Cs	Ba	Au	Hg	Tl	Pb
(1.69)	(1.53)	(1.37)	(1.25)	(1.15)	(1.06)

Univalent Anions

C	N	O	F	H
(4.14)	(2.47)	(1.76)	(1.36)	(2.08)
Si	P	S	Cl	
(3.84)	(2.79)	(2.19)	(1.81)	
Ge	As	Se	Br	
(3.71)	(2.85)	(2.32)	(1.95)	
Sn	Sb	Te	I	
(3.70)	(2.95)	(2.50)	(2.16)	

Ionization of weak electrolytes, or the rupture of a covalent bond
in the course of reaction, usually involves the intervention of coördina-
tion processes, as a result of which the amount of deformation of one or
both of the incipient ions is reduced. Coördination of a cationic (elec-
trophilic) center with a donor will reduce its deforming power owing
to the production of a more stable valence shell and to the dissemination
of the residual positive charge; coördination of an anion with an acceptor

[57] Pauling, *J. Am. Chem. Soc.*, **49**, 771 (1927).

center will reduce its deforming power and its deformability, since the residual negative charge and electron mobility are thereby diminished.

Diethylzinc is not appreciably ionized in the pure state and is a very poor conductor, but its conductivity is increased greatly by the addition of anhydrous ether, which is also a poor conductor. The effect of the ether can be attributed to the formation of etherates in which the deforming power of the cation $(R-Zn)^+$ is reduced. The alkyl anion, by coördination with unionized diethylzinc, can be converted also into a complex anion $[R_3Zn]^-$, of greatly diminished deformability. These relations are shown in the equilibria given below; a similar situation occurs in the usual ethereal solutions of Grignard reagents, giving rise to solvated molecules of R_2Mg, $RMgX$, and MgX_2, and to solvated ions such as $[RMg + 3Et_2O]^+$, $[R_3Mg + Et_2O]^-$, etc.

$$R_2Zn + 2Et_2O \rightleftarrows \begin{array}{c} R \\ \diagdown \\ R \diagup \end{array} Zn \begin{array}{c} OEt_2 \\ \diagup \\ \diagdown OEt_2 \end{array}$$

$$2 \begin{array}{c} R \\ \diagdown \\ R \diagup \end{array} Zn \begin{array}{c} OEt_2 \\ \diagup \\ \diagdown OEt_2 \end{array} \rightleftarrows \left[\begin{array}{c} OEt_2 \\ \downarrow \\ R-Zn\leftarrow OEt_2 \\ \uparrow \\ OEt_2 \end{array} \right]^+ \left[\begin{array}{c} R \\ | \\ R-Zn\leftarrow OEt_2 \\ | \\ R \end{array} \right]^-$$

Unionized complex   Complex cation   Complex anion
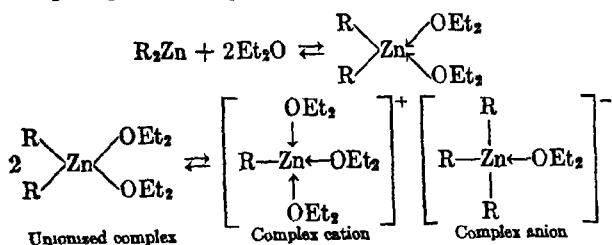
The introduction of an acceptor molecule such as BF_3 or $Al(OEt)_3$ can increase the ionization of an extremely weak acid such as ethyl alcohol.[29, 55] With reference to the ionization of acids Latimer and Rodebush[16] have made this statement: "It is doubtful if the hydrogen nucleus ever gets very far away from one or more electrons ... the ionization of acids, or extreme polarity of any compounds involving hydrogen, must be interpreted as due to the transfer of a hydrogen nucleus from one molecule to another, thus forming a complex ion." The action of BF_3 and similar acceptor molecules is shown in the following equations:

$$R-O-H + BF_3 \rightleftarrows \begin{array}{c} R \\ \diagdown \\ H \diagup \end{array} O-B \begin{array}{c} F \\ | \\ | \\ F \end{array} F$$

$$\begin{array}{c} R \\ \diagdown \\ H \diagup \end{array} O-B \begin{array}{c} F \\ | \\ | \\ F \end{array} F + R-O-H \rightleftarrows \left[\begin{array}{c} H \\ \diagup \\ R-O \\ \diagdown \\ H \end{array} \right]^+ \left[\begin{array}{c} F \\ | \\ R-O-B-F \\ | \\ F \end{array} \right]^-$$

Complex cation   Complex anion
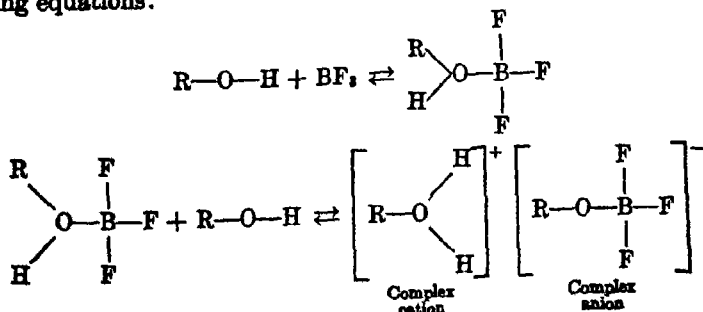
* Nieuwland and others, *ibid.*, **52**, 1018, 2892 (1930); **53**, 3835 (1931); **54**, 2017 (1932)

The ionization of acids and bases, and reversible chemical reactions in general, may be considered from the same standpoint. The completion of a chemical reaction involves merely the occurrence of an ionic displacement that is not reversible under the influence and conditions of the environment. This may be illustrated by the behavior of ethylsodium toward ether. The ether facilitates ionization, as it does with diethylzinc, but the relative reluctance of sodium to form a complex anion $[Na(C_2H_5)_2]^-$ leads to an attack of the ether by the alkyl anions. The resulting anionic complex is unstable and decomposes irreversibly, with the elimination of ethane and ethylene, to form the more stable

$$[C_2H_5:]^- + C_2H_5-O-C_2H_5 \rightarrow \left[C_2H_5 \rightarrow H \overset{\frown}{-CH_2} \overset{\frown}{-CH_2} -OC_2H_5\right]^-$$

$$\rightarrow C_2H_6 + C_2H_4 + [:O-C_2H_5]^-$$

$$[C_2H_5:]^- + C_2H_5-O-C_6H_5 \rightarrow [C_2H_5 \rightarrow H-CH_2-CH_2-O-C_6H_5]^-$$

$$\rightarrow C_2H_6 + C_2H_4 + [:O-C_6H_5]^-$$
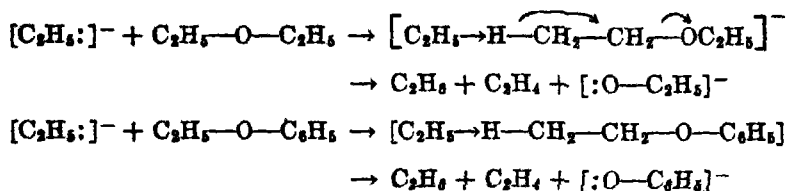
ethoxide anion. Ethylsodium attacks phenetole in a similar way and gives finally sodium phenoxide.

In covalent links of the hetero atoms in Groups I, II, and III, permanent acceptor activity will be limited primarily to the hetero atom itself but temporary acceptor activity may be conferred upon hydrogen in H—C links of an attached group by means of dynamic effects $(+I_d)$ and may be effective in the course of chemical reaction. These effects will be expected to occur particularly with hetero atoms having low polarizability and furnishing a potential cation of high deforming power, such as 3-covalent boron.* In saturated alkyl derivatives of boron the $+I_d$ effect is opposite in direction from the permanent polarization but might become important when the attachment of a donor center to the boron atom itself is impeded for steric reasons.

In the event that the atom attached to boron bears an unshared electron pair or a multiple covalent bond an electromeric shift can occur toward the boron atom, so that its electron deficiency will be diminished:

$R_2B\overset{\frown}{-O}-C_2H_5$ and $R_2B\overset{\frown}{-C}=C-R$. If a vinyl group is present, the

$+E$ effect of boron will result in a transfer of the electron deficit to the β-carbon atom (see below, hetero-enoid systems). If an aryl group is present, the effect in the ring is similar to that of a nitro or carboxyl

* The effective radius of the univalent boron cation has been estimated by Pauling [57] to be 0.35 Å; its deforming power should exceed that of any univalent cation except carbon (0.20 Å), nitrogen (0.25 Å), oxygen (0.22 Å), fluorine (0.19 Å), or hydrogen.

group and will cause a diminution in the ease of substitution and favor *meta* orientation.[59]

The electromeric shift of an unshared electron pair from the adjacent atom toward boron should diminish the acceptor activity of 3-covalent boron; the fact that BF_3 is a more powerful acceptor than $B(OC_2H_5)_3$ may be anticipated from the ability of the alkoxyl group to permit a greater electronic displacement ($-E$ toward boron) than fluorine does [60]

$$R-\overset{\frown}{O}-B > \overset{\frown}{F}-B.$$
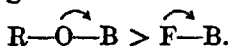
The stability of coördination complexes and the relative ease of ionic displacement within them are influenced by a variety of factors: the relative sizes of the donor and acceptor atoms, the spatial arrangement about the coördination center, the intervention of chelation and of resonance effects. The ability of an atom to act as an acceptor is affected by the electronic and steric characteristics of the attached groups, and the number of additional covalent links is limited by the maximum covalence rule. However, an atom directly combined to one or more hydrocarbon radicals rarely forms a stable complex in which its valence shell is expanded beyond an octet. A few exceptions have been cited previously (p. 1839), e.g., R_3PCl_2, $RAsCl_4$, $[CH_3—TeI_4]^-$, etc.; all these have two or more halogen atoms attached simultaneously with the organic groups, and even in this favorable situation the systems show a strong tendency to revert to an octet.

Some of the remarkable differences in behavior which are found in comparing elements of the first horizontal period with those in the second and higher periods of the same group can be explained on the basis of the ability of the larger atoms to exceed a covalence of four as a transient intermediate step in their reactions (p. 1838). Certain other divergences have been accounted for by the hypothesis that an atom which is capable of becoming 6-covalent can assume, although it does not usually do so, a plane space-distribution of its valences (angle 90°) in the 4-covalent state. Consequently such atoms would take part more readily in forming, and would give more stable, four-membered rings than the corresponding atoms of the first horizontal period. This may be the reason why boron, which is the only element in Group III incapable of becoming 6-covalent, is also the only element in that group to form trihalides that are not polymerized.[4] A four-membered chelate

[59] Seaman and Johnson, *ibid.*, **53**, 711 (1931).
[60] Yabroff, Branch, and Almquist, *ibid.*, **55**, 2935 (1933).

ring structure for aluminum chloride (Al_2Cl_6) would be essentially strainless on this assumption (p. 1876) but a similar ring for boron trichloride would have a large strain if boron is restricted to the tetrahedral configuration (angle 109°).

An explanation of the high catalytic activity of the chloride and alkoxides of aluminum, and of the almost complete absence of similar properties in the corresponding boron compounds, may be sought along the lines outlined above. Owing to the limited knowledge of the effects of coördination upon the donor and acceptor centers, and the recognized complexity of the factors governing the behavior of coördination complexes, it is to be expected that detailed predications cannot be made upon a firm basis at the present time.

Class 2 (Group IV). In the organic derivatives of silicon and the elements of the B-subgroup of Group IV (germanium, tin, and lead), the permanent polarization and the polarizability effects will be in the same direction as that in the compounds of elements in Groups I, II, and III. Owing to the larger effective nuclear charges of the hetero atoms in Group IV, and the presence of an octet of electrons in the valence shell of the hetero atom, the magnitude of the $-I_s$ effect and the polarizability will be smaller than that for the corresponding elements of the earlier groups: C—Si < C—Al < C—Mg < C—Na; C—Ge < C—Zn; C—Pb < C—Hg; etc. Within the fourth group the effects will increase toward the larger atoms:

$$C—Si < C—Ge < C—Sn < C—Pb$$

In these links the tendency to yield alkyl anions will be relatively small and their reactivity will depend largely upon the ability of the hetero atom to act as an acceptor by a temporary expansion of its valence shell beyond an octet. The stepwise dealkylation of the tetraalkyl derivatives by halogens, and the reactivity of these compounds in general, may be explained readily on this assumption.

$$(CH_3)_4Pb + Br_2 \rightarrow [(CH_3)_4Pb{\leftarrow}Br—Br] \xrightarrow[shift]{a,r-} (CH_3)_3Pb—Br + CH_3—Br$$

$$(CH_3)_4Pb + H—Br \rightarrow [(CH_3)_4Pb{\leftarrow}Br—H] \rightarrow (CH_3)_3Pb—Br + CH_4$$
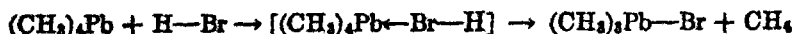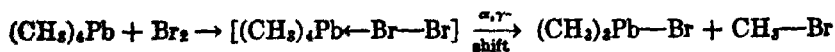
The observation that the cleavage of analogous unsymmetrical derivatives of mercury and lead by means of hydrogen chloride [61,62] gives

[61] Kharasch and Flenner, *ibid.*, **54**, 674 (1932).
[62] Gilman, Towne, and Jones, *Rec. trav. chim.*, **61**, 1054 (1932); *J. Am. Chem. Soc.*, **55** 4689 (1932).

the same products supports the notion that the mechanism of reaction is essentially the same in both cases (p. 519).

$$C_6H_5-Hg-C_2H_5 + HCl \rightarrow C_2H_5-HgCl + C_6H_6$$

$$(C_6H_5)_2Pb(C_2H_5)_2 + 2HCl \rightarrow (C_2H_5)_2PbCl_2 + 2C_6H_6$$
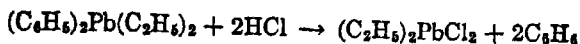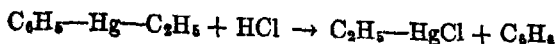
When electromeric effects can intervene these hetero atoms may exert a $+T$ effect which will oppose the I_s effect, and the situation is similar to that outlined for boron. Consequently, these hetero atoms may be expected to exert a *meta* directive influence (as well as *ortho-para*) in aromatic substitution reactions. The nitration of phenyl-germanium trichloride $(C_6H_5-GeCl_3)$ with fuming nitric acid at low temperatures has been found to give 58 per cent of *meta* and 42 per cent of *para* substitution;[63] under similar conditions phenylboric acid, $C_6H_5-B(OH)_2$, gives 85 per cent of *meta* and 15 per cent of *ortho* substitution.[59] Using acetic anhydride and a minimum excess of nitric acid, phenylboric acid gives almost exclusively *ortho* substitution. This reversal of orientation may be attributed to an opposing, secondary effect in an addition compound of phenylboric acid with acetic anhydride.[60] When the weak $+T$ effects of boron and germanium are reinforced by an appropriately situated methyl group, as in the *p*-tolyl derivatives, nitration occurs entirely in the *meta* position (with reference to Ge or B).

The sharp difference in the behavior of the C—C link and the links C—Si, C—Sn, and C—Pb is due to the inability of carbon to expand its valence shell to a decet. The attack of a donor molecule *must* occur through an H—C link of a substituent group. The action of chlorine on neopentane results in a substitution process in one or more of the methyl groups (dehydrogenation) and not in a replacement of the alkyl group (dealkylation) such as occurs with tetramethylsilicane.

$$(CH_3)_3C-CH_3 + Cl_2 \rightarrow [(CH_3)_3C-CH_2-H \leftarrow Cl-Cl]$$

$$\xrightarrow[\text{shift}]{\alpha,\gamma} (CH_3)_3C-CH_2Cl + HCl$$

$$(CH_3)_4Si + Cl_2 \rightarrow [(CH_3)_4Si \leftarrow Cl-Cl] \xrightarrow[\text{shift}]{\alpha,\gamma} (CH_3)_3SiCl + CH_3Cl$$
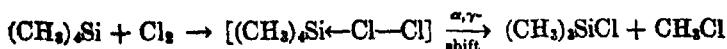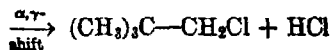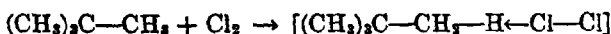
C—C and C—H Bonds. In unsymmetrically substituted C—C links the permanent polarization is exceedingly small except where powerful effects are introduced by the presence of active hetero atoms in adjacent links. Nevertheless, the behavior of aliphatic hydrocarbon systems in-

[63] Shelton, Washington Meeting, Am. Chem. Soc. (1933).

dicates that definite directive influences are operative in the course of reactions.

In saturated systems only inductive displacements (I_s and I_d effects) are possible. The *relative* effects of the alkyl groups are summarized below:

$+I_s$ H—> CH₃—> Primary> Secondary> Tertiary
                       groups   groups   groups
                 Relative inductive electron-attraction

$-I_s$ *and*   Tertiary> Secondary> Primary> CH₃—> H—
$\pm I_d$ groups   groups   groups
             Relative inductive electron-release and relative polarisability
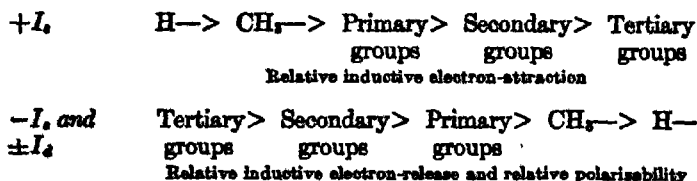
The small permanent polarization of the link H—C will be diminished by the replacement of hydrogen by an aliphatic radical, owing to the essential equivalence of the effective nuclear charges in the atoms of the C_a—C link. Alkyl groups will have a permanent effect of electron-release ($-I_s$) relative to hydrogen, and the relative magnitude of this effect will increase as the number of hydrogens attached to C_a diminishes.

In dealing with a specific reaction it is essential to formulate a definite reaction mechanism and to take into account the type of displacement that will facilitate or impede the necessary electronic change (i.e., the electrical demand of the reagent). Thus, in a series of alcohols the relative proton-escaping tendency in the link H—OR increases as the relative electron-attraction of the R groups increases, since a proton will escape more readily as the electron density in the OH residue is diminished: R—←—O—H. Consequently the proton-escaping tendency of the alcohols will be expected to follow the trend of $+I_s$ effects; this is confirmed by experimental observations, which give the sequence:

H—O—H> CH₃—O—H> Primary> Secondary> Tertiary
                           alcohols   alcohols   alcohols

The tendency of these hydroxylic compounds to form dimers of the type H—OH←—OH₂ by intermolecular association falls off in the same direction and must be attributed largely to the diminishing acceptor activity of the active hydrogen.

The capacity of the oxygen atom to act as a donor will be enhanced as electron-release by the R group increases since this augments the mobility of the unshared electrons in its valence shell. Consequently the tendency of the alcohols to form oxonium salts, or complex cations in general, should increase in the order: primary < secondary < tertiary alcohols. The oxonium complexes derived from the simple alcohols are highly active systems and their behavior involves a consideration of

alternative courses of reaction (see pp. 1866 and 1867 for example): formation of olefins, alkylation reactions, simple ionization, etc.

Coördination of the oxygen atom with a proton (or other acceptor) will result in a strong tendency to withdraw the electron pair of the link $C\!-\!OH_2{}^+$. The mobility in the oxonium complex will allow a suitable approach of the donor center and the potential alkyl cation; reaction I is the result of an α,γ-shift within the complex. Reaction II requires the intervention of polarizability effects, which determine the tendency of an alkyl residue to separate from the complex as a *free* cation. The alkyl cations will be extremely unstable owing to the electron deficit (open sextet) of the α-carbon atom, and will revert to a more stable configuration by ejecting a proton and forming an olefin (or a cyclic structure). The effect of substituents upon the course of rearrangements occurring in the dehydration of alcohols, and in many similar processes,

$$C_2H_5\!-\!O\!\rightarrow\!H\!-\!X \xrightarrow{\ \alpha,\gamma\text{-shift}\ } C_2H_5\!-\!X + H_2O$$
$$\overset{\text{H}}{\underset{}{}}\qquad\qquad\qquad\qquad\qquad\qquad\text{I}$$
$$[C_2H_5\!-\!OH_2]^+X^- \rightarrow [C_2H_5]^+ \rightarrow C_2H_4 + H^+$$
$$\text{II}$$
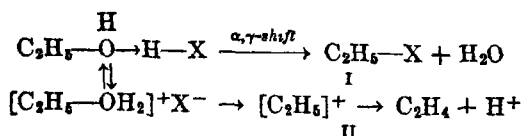
has been treated with remarkable success by Whitmore [44] from the standpoint of the electronic configurations of the potential alkyl cations (p. 1018).

Interpretation of the behavior of organic systems containing an aryl group directly attached to a reactive center ($C_6H_5\!-\!X$), or separated from it by an aliphatic system ($C_6H_5\!-\!CH_2\!-\!X$, $(C_6H_5)_2CH\!-\!X$, $C_6H_5\!-\!CH\!=\!CH\!-\!X$, etc.), involves a consideration of electromeric effects of aromatic groups and their interaction with other effects in the system and in the reagent. Experimental evidence supports the view that aryl groups can exert dynamic effects of either sign and are capable of activating electron-attraction or electron-release, depending upon the nature of the reaction.

The ability of aryl groups to confer upon the attached atom an increased tolerance for an electrical charge of *either* sign is intimately associated with certain qualities represented by the term "aromaticity." The enhanced stability of the phenoxide and benzyl anions relative to their aliphatic analogs, and that of triarylmethyl cations relative to all other hydrocarbon cations, illustrate the intervention of dynamic effects of opposite sign. The dual polarizability effects may be attributed to the ability of the aromatic nucleus, by means of electromeric displacements in directions determined by the sign of the charge, to bring about

a distribution of this charge primarily at the *para* and the *ortho* positions, and thence, by secondary inductive displacements, also to the *meta* positions, so that the residual atomic charge will ultimately be distributed over the entire system.[7] Owing to the large number of possible positions for a residual charge the stability of the ions is enhanced. Influences of this kind (resonance effects) are significant in a number of organic compounds containing multiple bonds; the distinctive feature of aromatic systems (p. 1929) is their ability to compensate an electronic deficit or an electronic excess with nearly equal facility.

Class 3 (Groups V, VI, VII). The single links encountered most frequently in organic reactions are those of carbon to nitrogen, oxygen, and the halogens. The distinctive feature of the electronic configurations of these links is the presence of one or more unshared electron pairs in the valence shell of the hetero atom.

Owing to the higher effective nuclear charges of these hetero atoms relative to carbon, a permanent inductive displacement toward the hetero atom will take place in the typical single bonds ($+I_s$ effect with reference to carbon). The magnitude of the permanent polarization, and the deforming power of the hetero atom as a potential anion, change in a regular manner with the position of the hetero atom in the periodic table. These characteristics increase as the effective nuclear charge of the hetero atom becomes larger, i.e., as the atom moves to the right within each period: $N < O < F, P < S < Cl$, etc. The diminution of effective nuclear charge with increasing atomic radius causes the inductive effects to decrease within each group, in passing to the higher periods: $F > Cl > Br > I, N > P > As > Sb > Bi$, etc.

The deformability of the hetero atoms and the tendency of their unshared electron pairs to engage in electromeric effects (or donor activity) are properties related to the mobility of the electrons in the valence shell. These characteristics have an inverse relationship to the polarization effects of the hetero atoms and increase in the opposite direction. The mobility of unshared electron pairs decreases in going from left to right within the periods: $N > O > F$; $P > S > Cl$; etc. Within each group the electron mobility increases in passing into the higher periods $-I > -Br > -Cl > -F; -Sb > -As > -P > -N$; etc. It should be noted, however, that the mesomeric (permanent) polarization effects of the halogen atoms follow the order $-F > -Cl > -Br > -I$.[*]

In saturated aliphatic systems donor activity is restricted to the hetero atom itself but in unsaturated systems may be transmitted through an intervening chain (see hetero-enoid systems). Many of the

* For a concise discussion of the four distinct polar effects of the halogen atoms, see Bird and Ingold, *J. Chem. Soc.*, 927 (1938).

typical reactions of the hetero atoms in Groups V, VI, and VII occur through coördination with an acceptor center of the reagent; a few examples are indicated briefly in the following equations:

$$R_3N + H—Br \rightarrow [R_3N \rightarrow H—Br] \rightarrow [R_3N—H]^+ \quad Br^-$$

$$R—OH + H—Br \rightarrow \left[\begin{matrix} H \\ | \\ R—O \rightarrow H—Br \end{matrix} \right] \rightarrow R—Br + H_2O$$

The permanent polarization effects of these hetero atoms will tend to create an active center of low electron density (acceptor activity) in the system. The introduction of chlorine or ethoxyl in a hydrocarbon chain increases the electron deficit of the carbon to which it is linked (relative to H—C). This deficit is overcome in part by secondary inductive displacements in adjacent links, and by a similar mechanism is transmitted with successive diminutions to more distant atoms in the system.

$$\overset{a}{X} \leftarrow \overset{b}{C} \leftarrow H \qquad \overset{a}{X} \leftarrow \overset{b}{C} \leftarrow \overset{c}{C} \leftarrow H$$

$$X—C—H + Cl_2 \rightarrow [X—C—H \leftarrow Cl—Cl] \rightarrow X—C—Cl + HCl$$

As a result of the inductive effects of chlorine and oxygen, alkyl halides and ethers will react with donor reagents more readily than the parent hydrocarbons, and will undergo direct substitution preferentially on the α-carbon atom. The presence of two hetero atoms in close proximity in an organic system will lead to an enhanced reactivity of the system; the result of a reaction in these systems will involve the intervention of polarizability effects, steric factors, etc.

$$CH_3—CHCl—OC_2H_5 \begin{array}{l} \overset{KOH}{\nearrow} \quad CH_2{=}CH—OC_2H_5 + KCl \\ \\ \underset{RMgX}{\searrow} \quad CH_3—CH \begin{array}{l} {\diagup} OC_2H_5 \\ {\diagdown} R \end{array} + MgX_2 \end{array}$$

$$CH_3—CHOH—OC_2H_5 \rightleftarrows CH_3—CH{=}O + C_2H_5—OH$$

The inductive effects of halogens and hydroxyl and alkoxyl groups result in an increase in the strength of the acid when these substituents are introduced into an aliphatic system containing the carboxyl group. By means of a logarithmic function based upon the ionization constants of a series of substituted acids, Derick [65] developed certain generalizations concerning the influence of the substituent. If the inductive effect of a halogen atom in the α-position is taken as unity, its influ-

[65] Derick, J. Am. Chem. Soc., 33, 1152, 1181 (1911).

ence in the β-, γ-, and δ-positions in the isomeric acids is $\frac{1}{3}$, $\frac{1}{9}$, and $\frac{1}{27}$, respectively. More recently Hixon, Johns, and their collaborators[46] have shown that the polar properties of an extended series of organic compounds including R—OH, R—CO$_2$H, R—CH$_2$—CO$_2$H, R—NH$_2$, R—HgX, and others, can be expressed as an exponential function of an arbitrary number representing the "electron-sharing ability" of R (provided that R does not contain polar groups).

In the saturated aliphatic derivatives of nitrogen, oxygen, and fluorine, the participation of the hetero atom in coördination processes is limited to donor activity since their valence shells are restricted to an octet. In appropriate unsaturated and aromatic structures the donor activity may be transmitted through a chain of covalent bonds (see hetero-enoid systems). Atoms of the second and higher periods can expand their valence shells beyond an octet, although they rarely form stable organic compounds with an enlarged valence shell. The capacity of these atoms to expand their valence shells, and the instability of the resulting configuration, suggest that under suitable conditions the mechanism of their reactions may involve acceptor activity of the hetero atom. This premise may be used as the basis for an explanation for certain distinctive reactions of the atoms within a given periodic group (see pp. 1838–1840); this view is supported by a good deal of indirect evidence.

Owing to the fact that the permanent polarization effects of these hetero atoms enhance the acceptor activity of adjacent atoms, it seems probable that acceptor activity of the hetero atom itself is associated largely with polarizability factors. It is observed that the general tendency to expand the valence shell beyond an octet is more prominent in the larger atoms and follows the trend of polarizability effects. The greater capacity of iodine to behave as a relatively "positive" atom in its reactions affords an illustration of this trend.[47]

$$R\text{—Cl (and R—Br)} + H\text{—OH} \rightarrow [R\text{—Cl} \rightarrow H\text{—OH}] \xrightarrow[\text{shift}]{a,\gamma} R\text{—OH} + HCl$$

$$R\text{—I} + H\text{—OH} \xrightarrow{\text{Normal}} R\text{—I} \rightarrow H\text{—OH} \rightarrow R\text{—OH} + HI$$

$$R\text{—I} \leftarrow OH_2 \xrightarrow[\text{shift}]{a,\gamma} R\text{—H} + HOI$$
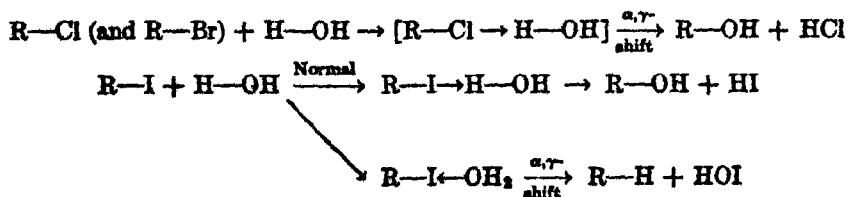
One may anticipate that the influence of substituents upon the normal reactions will be reversed for "abnormal" reactions, and this appears to be true. The reactivity of n-propyl chloride in a typical

[46] Hixon, Johns, and collaborators, *ibid.*, **49**, 1786 (1927); **50**, 168 (1928); **53**, 4367 (1931); **54**, 3971 (1932); *J. Phys. Chem.*, **34**, 2218, 2226 (1930).

[47] Nicolet and collaborators, *J. Am. Chem. Soc.*, **43**, 2081 (1921); **49**, 1796, 1801, 1806 (1927).

metathetical reaction is much greater than that of isopropyl chloride, but in an "abnormal" reaction, such as the formation of alkyl chlorides from the iodides and mercuric chloride, isopropyl iodide reacts at least fifty times more rapidly than n-propyl iodide.[68]

A comparison of certain reactions of the alkyl derivatives of arsenic, antimony, and bismuth with those of the corresponding elements of Group IV (tin, germanium, and lead) affords an interesting illustration of the influence of polarizability effects upon chemical behavior. The formation of salts of the type $[(CH_3)_4Sb]^+Cl^-$ may be taken as a characteristic reaction of the elements of Group V (B-subgroups); the removal of the alkyls by means of halogens is a typical reaction for the elements of Group IV, and earlier groups. The first reaction occurs readily when the alkyl derivatives of arsenic or antimony are treated with an active alkyl halide, but the alkyl bismuthines behave differently. Trimethylbismuthine reacts with methyl iodide upon warming but it yields ethane and methylbismuth diiodide instead of a bismuthonium salt.[69] In this respect the bismuthine resembles alkyl derivatives of zinc and mercury rather than those of arsenic and antimony.

$$R_3Sb \rightarrow CH_3—I \rightarrow [R_4Sb]^+ I^-$$
$$R_3Bi \leftarrow I—CH_3 \rightarrow R_2BiI + R—CH_3$$
$$\downarrow \text{Excess } CH_3I$$
$$R—BiI_2 + R—CH_3$$
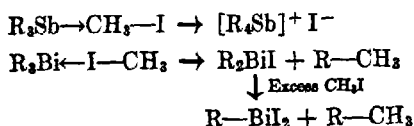
The anomalous behavior of the bismuthine suggests that the nature of the reaction is altered by a difference in the relative polarizability effects of antimony and bismuth with reference to iodine. The behavior of the stibine is like that of amines with alkyl halides, but that of the bismuthine probably involves coördination of the bismuth and iodine atoms. This process facilitates the separation of an alkyl anion from the bismuthine and its combination with the positively polarized alkyl group of the alkyl halide.

In their behavior toward the free halogens, there is a certain resemblance between the alkyl derivatives of the elements of Group V and Group IV. Phosphorus, arsenic, and antimony form halides of the type R_3PCl_2, R_2PCl_3, and $RPCl_4$, which are generally stable enough to be isolated. Upon warming, they tend to undergo dealkylation and yield products similar to those obtained by the action of halogens on the alkyl derivatives of the elements of the earlier groups. The normal

$$R_3As + Cl_2 \rightarrow R_3AsCl_2 \xrightarrow{\text{Heat}} R_2AsCl + R—Cl$$
$$R_4Ge + Cl_2 \rightarrow [R_4GeCl_2] \longrightarrow R_3GeCl + R—Cl$$
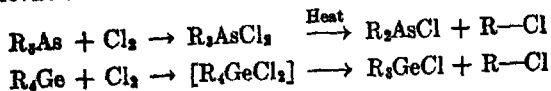
[68] Nicolet and Potts, *ibid.*, **50**, 212 (1928).
[69] Breed, *Ann.*, **82**, 106 (1852); Dünhaupt, *Ann.*, **92**, 371 (1854); Marquardt, *Ber.*, **20**, 1516 (1887); **21**, 2035 (1888).

form of the pentavalent chlorides of the fifth group elements is probably that in which the central atom is 5-covalent; their stability relative to the corresponding compounds of the earlier groups is due to the higher effective nuclear charge of the central atom. The bismuth alkyls are decomposed directly by halogens, but the triaryl derivatives form stable crystalline dihalides of the type R_3BiCl_2.

Hydrolysis of the 5-covalent dihalides gives oxygen compounds in which the central atom reverts to an octet. The central atom is undoubtedly only 4-covalent in the normal forms of the arsine oxides, arsinic, and arsonic acids (and in the corresponding derivatives of phosphorus and antimony).

Multiple Covalent Bonds. When there are not enough electrons in a molecule to provide each atom with its stable octet by the process of forming single covalent bonds, two contiguous atoms may share a second or third pair of electrons. The extent of this sharing is by no means so complete or unambiguous as in the single bond; furthermore, the ability to share a second or third pair is almost entirely limited to carbon, nitrogen, and oxygen.[1] This property appears to be associated with the helium configurations of these atomic kernels; the "double bonds" of phosphorus, sulfur, and other elements of Groups V, VI, and VII, outside the first period, are usually coördinate links. The behavior of systems represented for convenience by formulas such as SiO_2, $R\!-\!SiO_2H$, $R\!-\!GeO_2H$, etc., is consistent with the view that these substances are actually polymerized in the normal state and would be more accurately represented as "giant" molecules. In the case of boron in the alkyl boric oxides, $R\!-\!B\!=\!O$, the polymerization gives a cyclic trimer analogous to the trimeric aldehydes.[70]

"Giant" molecule (linear polymer)

Cyclic trimer
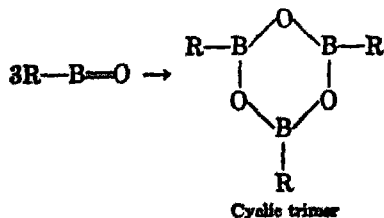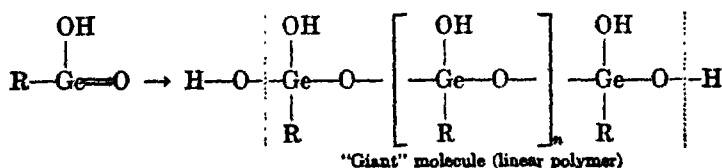
[70] Johnson and Snyder, Organic Chemistry Symposium, Rochester (1935); *J. Am. Chem. Soc.*, **69**, 107 (1938); see, also, Kinney and Ponts, *ibid.*, **58**, 197 (1936).

Lewis [1] pointed out that the sharing of more than one electron pair by two atoms represents, because of the mutual repulsion of the nuclei, a point of weakness or condition of strain in the molecule, and this tends to keep the system from settling into a state of high stability and low electron mobility. Furthermore, the formation of a multiple bond is accompanied by a diminution of the internuclear distance; this amounts to about 10 per cent for a double bond and 20 per cent for a triple bond. He drew the conclusion that the properties of substances with multiple bonds are due to an extent of sharing of two or three electron pairs which is probably less than that indicated in the usual graphic formulas; but the sharing must be assumed to have some physical reality in order to account for the existence of geometrical isomerism and similar steric phenomena.

True covalent multiple bonds of the types A=B and A≡B are regarded as capable of existing in an inactive form in which both atoms have a valence octet, and a reactive form in which electromeric displacement of an electron pair creates an electron deficit in the valence shell of one atom (an open sextet) and an increased electron mobility in the other.[n] The typical states of a double and triple bond may be represented in the following manner (see, also, p. 1846):

$$\overset{\frown}{A}=B \quad \rightleftarrows \quad A=B \quad \rightleftarrows \quad A\overset{\frown}{=}B$$

$$\overset{\frown}{A}\equiv B \quad \rightleftarrows \quad A\equiv B \quad \rightleftarrows \quad A\overset{\frown}{\equiv}B$$

Activated state   Normal state   Activated state
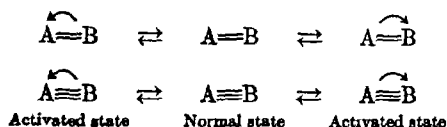
The symmetrical active forms that would result from a rupture of the binding pair would give each atom only seven electrons. It seems quite improbable that this mode of activation is significant in the typical reactions of unsaturated systems. Lewis has expressed the view that the pairing of electrons ("rule of two") is even more fundamental than the octet rule, and that a substance containing unpaired electrons would be far more reactive than ethylene actually is.

The active forms are assumed to be extremely mobile and capable of only momentary existence, so that their concentration is always small; owing to the reversibility of the electromeric displacement there is equilibrium between the active and inactive forms. If A and B are different atoms, then, in the activation process, the atom having the higher effective nuclear charge will tend to retain the electron pair and the atom having the smaller nuclear charge will become the deficient atom in the active form. The direction of addition of unsymmetrical addenda

[n] Carothers, J. Am. Chem. Soc., 46, 2226 (1924).

(HX, RMgX, etc.) will be highly selective and will be determined essentially by the electronic configuration of the dominant active form.[71]

$$R_2C \overgroup{=\!=} O, \quad R_2C \overgroup{=\!=} NH, \quad R \overgroup{-\!C} \equiv N$$

Since the two atoms remain attached by one covalent link (two in the triple bond) the deficient atom never gets far away from the displaced electron pair.

The electromeric displacement will affect the remaining covalent links (shared electron pairs) and the mobility of unshared electron pairs in the entire system. The displacement $A \overgroup{=\!=} B$ will increase the donor activity of B and will have a dynamic effect of electron-release in the covalent links of B with a substituent. This dynamic influence can be effective in chemical reactions only through unshared electron pairs or other multiple bonds in the substituent; it can interfere with the acceptor activity of B or its substituents only by promoting a different course of reaction. Electron withdrawal by B will increase the acceptor activity of A and the atoms or radicals attached to A, and will facilitate their reactivity toward a donor reagent. A carbonyl group will increase the proton-escaping tendency, and diminish the availability of electrons, in the system into which it is introduced. Thus, acetic acid is a stronger acid than methyl alcohol, and acetamide is a weaker base than methylamine.

It must be noted that the electron attraction of a carbonyl group (or other multiple bond) does not impede the separation of an anion from the α-carbon atom in a system such as $Cl\!-\!CH_2\!-\!A \overgroup{=\!=} B$; in fact, it can facilitate the elimination of the potential anion (i.e., replacement by another) by an indirect process such as that indicated below.

$$R-C\!\!\begin{array}{c} O \\ CH_2Cl \end{array} + [:OEt]^- \longrightarrow \left[R-\overset{O^-}{\underset{\underset{Cl}{CH_2}}{C}}-OEt \right] \longrightarrow \bar{Cl} + \left[R-\overset{O}{\underset{CH_2}{C}}-OEt \right] \longrightarrow R-C\!\!\begin{array}{c} O \\ CH_2OEt \end{array}$$

$$+ :NR_2 \longrightarrow \left[R-\overset{O}{\underset{\underset{Cl}{CH_2}}{C}}-\overset{+}{N}R_2 \right] \longrightarrow \bar{Cl} + \left[R-\overset{O}{\underset{CH_2}{C}}-NR_2 \right]^+ \longrightarrow \left[R-C\!\!\begin{array}{c} O \\ CH_2NR_2 \end{array} \right]$$
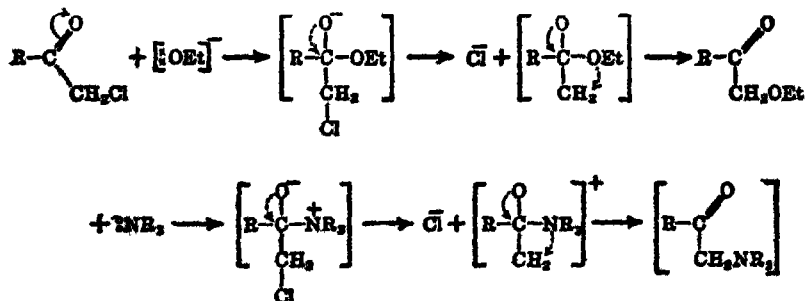
In an α-halogenated ketone the electromeric effect of oxygen, and the transmitted inductive effect due to the halogen, enhance the electron deficit of the carbonyl carbon. Under appropriate conditions the defi-

cient carbon will coördinate with a donor, such as trimethylamine or an ethoxide anion, to form a neutral complex or a complex anion. In either case the oxygen has a high residual negative charge resulting from the acquisition of the electron pair of the double bond; the electromeric effect will tend to be reversed and the elimination of halide ion from the complex is facilitated. As this occurs the entering donor center becomes linked to the α-carbon.* The effect of the reversed electromeric displacement will diminish rapidly as additional CH_2 groups intervene between the halogen and the carbonyl group.

In many instances, reactions of the typical systems, such as $C=N$, $N=O$, and $C=O$, involve a preliminary coördination in which nitrogen or oxygen acts as a donor; this process facilitates withdrawal of the electron pair from the adjacent atom in the link and also increases the mobility of the potential donor center in the reagent molecule. The reactions of these systems with Grignard reagents [31] has been interpreted in this way (p. 1880); the normal reactions involve an α,γ-migra-

$$CH_3—Mg—Br + R_2C \overset{\frown}{=} O \rightarrow R_2C \overset{\frown}{=} O \rightarrow Mg—Br \xrightarrow[\text{shift}]{\alpha,\gamma^-} R_2C—O—Mg—Br$$

(with OEt$_2$ groups attached to Mg)
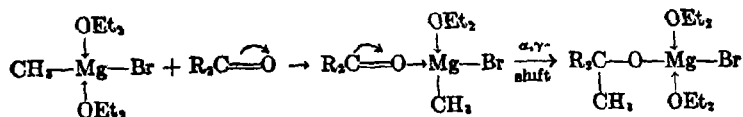
tion of the potential organic anion to the deficient atom of the unsaturated system. The fact that ethylenic and acetylenic bonds do not react with Grignard reagents is indicative of the importance of unshared electron pairs of nitrogen and oxygen in the activation mechanism.

The extent of electromeric displacement in the systems $A=B$ and $A\equiv B$, under a given set of conditions, will increase as the opposed nuclear charges become greater and for a given compound will be influenced by such factors as the temperature, concentration, characteristics of the medium, catalysts, etc. This permits an explanation of the difference in reactivity of different multiple bonds under the same conditions, and of the same multiple bond under different conditions.[71] The susceptibility of a system to perturbing influences of substituents (internal factors) and of external factors will increase as the difference in opposed nuclear charges becomes smaller, and therefore reaches a maximum when A and B are the same element. In systems such as $R_A—HC=CH—R_B$ and $R_A—N=N—R_B$, the configuration of the active form will depend upon the relative influences of the substituents R_A and R_B, and both

* The formulas given above do not take into account the intervention of the cation, the possibility of chelation within the complex, and other influences which may play an important part in certain cases. It is obvious that if the group R can itself be eliminated as an anion, as in $Cl—CO—CH_2Cl$, the complex anion indicated above would yield an ester instead of the ethoxy ketone.

active forms may result. In general these systems exhibit a definite orientation in their addition reactions; the process of activation involves the interaction of polarization and polarizability effects of the substituent and the multiple link under consideration.

The inductive effect (electron-attraction) of the hetero atoms, nitrogen, oxygen, and halogens, in their links with carbon, will facilitate a reaction involving the enhanced acceptor activity of 1-covalent hydrogen in the attached system, but their electromeric effects of electron-release ($-E$) will foster a dynamic displacement toward the β-carbon atom (see hetero-enoid systems). Consequently, in the orientation of addition reactions only the $-E$ effect will be significant. The inductive effect of alkyl groups (electron-release relative to hydrogen) will also be of assistance in the orientation process. For similar reasons the inductive electron-release of an atom such as boron will be opposed by its capacity to exert a dynamic effect in the opposite direction. The anticipated orientation in the addition of an unsymmetrical reagent is indicated below for several ethylenic systems.

$$Br—CH{=}CH_2 + H—X \rightarrow Br—CHX—CH_3$$

$$CH_3{\rightarrow}CH{=}CH_2 + H—X \rightarrow CH_3—CHX—CH_3$$

$$Br—CH_2—CH{=}CH_2 + H—X \rightarrow Br—CH_2—CHX—CH_3$$

$$RO—CH{=}CH_2 + H—X \rightarrow RO—CHX—CH_3$$

$$O{=}C—CH{=}CH_2 + H—X \rightarrow O{=}C—CH_2—CH_2X$$
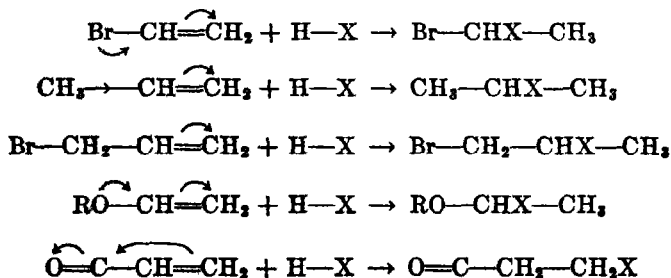
Recent work of Kharasch and his associates [72] supports the notion that the normal addition of hydrogen bromide to the first three of these systems follows the course indicated, but it was observed that the presence of minute amounts of active substances such as peroxides can suffice to bring about the formation of a large proportion of the isomeric product. The fact that thiophenols usually add exclusively in a direction opposite to that of most reagents appears to be due to the presence of traces of disulfides, which have a peroxide effect; when these impurities are rigorously excluded the mode of addition is the normal one.[73]

The orientation of addition reactions of unsymmetrical olefins and acetylenes involves the relative effects of electron-attraction and elec-

[72] Kharasch and collaborators, *J. Am. Chem. Soc.*, **55**, 2468, 2521, 2531 (1933); **56**, 712, 1212, 1243, 1425, 1443 (1934); **58**, 57 (1936); *J. Soc. Chem. Ind.*, **54**, 939 (1935).

[73] Posner, *Ber.*, **38**, 646 (1905); Ashworth and Burkhardt, *J. Chem. Soc.*, 1791 (1928); Carothers, *J. Am. Chem. Soc.*, **55**, 2008 (1933); Jones and Reid, *ibid.*, **60**, 2453 (1938).

tron-release in the hydrocarbon substituents. In the aliphatic series the relative electron-attraction diminishes as the number of hydrogen atoms on the α-carbon is reduced: $CH_3 > C_2H_5 >$ primary $>$ secondary $>$ tertiary groups. All these groups have an effect of electron-release relative to H—C; consequently the principal active configuration of an unsaturated hydrocarbon will be that in which the electromeric displacement occurs toward the carbon bearing the larger number of hydrogen atoms. The addition of reagents such as H—X and X—OH will tend to occur in such a way that the donor center of the reagent unites with the carbon atom bearing the larger number of alkyl groups (cf. Markownikoff's rule). The predominating active forms of several hydrocarbon systems are indicated below; the observed (normal) addi-

$$CH_3\!-\!C\overset{\frown}{=\!=}CH\!-\!CH_3 \quad CH_3\!-\!C\overset{\frown}{=\!=}CH \quad C_6H_5\!-\!CH=\overset{\frown}{=}CH_2 \quad C_6H_5\!-\!C\overset{\frown}{=\!=}C\!-\!H$$
$$\overset{|}{C}H_3$$

$$CH_3\!-\!CH_2\!-\!CH=\overset{\frown}{=}CH\!-\!CH_3 + H\!-\!Br \rightarrow \begin{cases} CH_3\!-\!CH_2\!-\!CHBr\!-\!CH_2\!-\!CH_3 \\ \text{(78 per cent)} \\ CH_3\!-\!CH_2\!-\!CH_2\!-\!CHBr\!-\!CH_3 \\ \text{(22 per cent)} \end{cases}$$
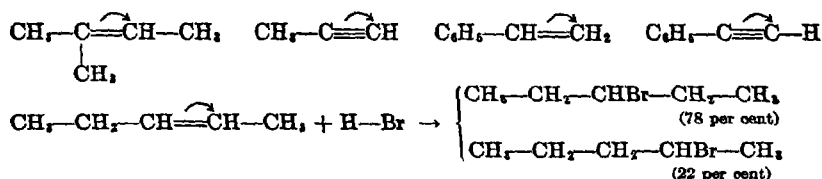
tions of these systems support the anticipated selective activation. In the case of pentene-2, containing the two similar alkyl radicals methyl and ethyl, it was found that addition of hydrogen bromide gave a mixture of the isomeric alkyl bromides in which the normal product predominates, i.e., 78 per cent of 3-bromopentane to 22 per cent of 2-bromopentane.[74] In the addition reactions of arylated systems such as styrene and phenylacetylene, the phenyl group has an effect of electron-release relative to hydrogen; the electron-attraction of aryl groups facilitates substitution in the unsaturated side chain but does not contribute to the orientation of addition reactions. Triphenylethylene, for instance, forms with bromine an unstable addition product which decomposes readily to give triphenylvinyl bromide.

Different opinions are held concerning the intimate mechanism of addition reactions of the olefins. Ingold and Robinson consider the addition of hydrogen halides, halogens, and other typical reagents to be initiated by the union of a positive atom (acceptor or electrophilic center) of the addendum with the atom acquiring the unshared electron pair in the activated form of the olefin. Carothers [71, 75] has expressed the view that the first step is the completion of the electron deficit in the valence shell of the positive atom in the active form of the olefin, by

[74] Lucas and Moyse, *J. Am. Chem. Soc.*, **47**, 1459 (1925).
[75] Carothers and Berchet, *ibid.*, **55**, 1628 (1933).

means of an unshared electron pair of the addendum. So far as the addition reactions of simple olefins are concerned, the same final product would be anticipated from either view of the intimate mechanism of the process.

$$
\begin{array}{c}
\text{H} \\
\text{HC} \rightarrow \text{H} \\
| \quad \quad | \\
\text{HC}^* \quad \text{Br} \\
\text{R}
\end{array}
$$

$$
\begin{array}{c}
\text{H} \\
\text{H}-\text{C} \\
\quad \; \| \\
\text{H}-\text{C} \\
\quad \; | \\
\text{R}
\end{array}
+
\begin{array}{c}
\text{H} \; (\delta+) \\
| \\
\text{Br} \; (\delta-)
\end{array}
$$

$$
\begin{array}{c}
\text{H} \\
\text{HC}: \text{H} \\
| \quad \; | \\
\text{H}-\text{C}-\text{Br} \\
| \\
\text{R}
\end{array}
$$

$$
\begin{array}{c}
\text{H} \\
\text{H}-\text{C}-\text{H} \\
| \\
\text{H}-\text{C}-\text{Br} \\
\text{R}
\end{array}
$$
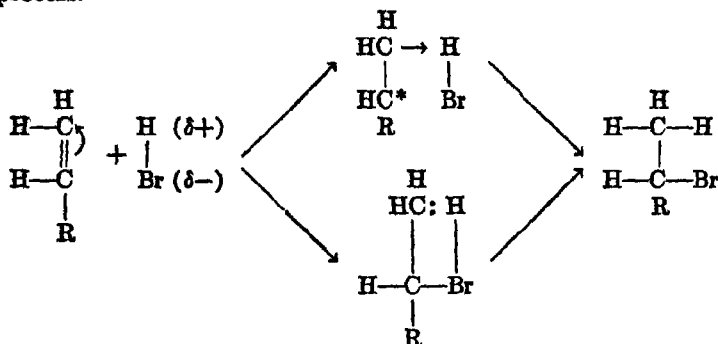
Olefins and carbonyl compounds exhibit a rather definite contrast in their behavior toward chemical reagents (p. 632). Olefins add halogens, halogen acids, nitric and sulfuric acids, ozone, per-acids (R—CO_3H), and oxyhalogen acids, which are electrophilic reagents. Olefins do not react in general with nucleophilic reagents such as ammonia and its derivatives (RNH_2, NH_2OH, NH_2—NH_2), alcohols, alkalies, cyanides, and Grignard reagents. The carbonyl compounds, on the other hand, undergo reaction with the latter group of reagents. Largely because of such differences in the facile type of reactivity, Ingold and Robinson regard the olefins as nucleophilic (donors) relative to the reagents, and the carbonyl compounds as electrophilic (acceptors). This view appears to rest upon the assumption that the ability of oxygen to contribute to the activation process by means of its unshared electron pairs is trivial in relation to the reactivity of the carbonyl compounds; but in the activation of an olefin the electronic excess of one carbon atom is assumed to be responsible for the ultimate acceptor activity of the other.

Robinson [78] considers that, for an equal degree of electronic excess or defect, any carbon atom is much more active in the former condition and that the extent of electromeric change ("activation level") normally found in olefins is not sufficient to initiate reaction with a donor reagent. Addition reactions are regarded in this way: "When the anionoid (donor) center is an olefin which begins to donate electrons to an external molecule, the electromeric change will naturally take a further step, and an adequate defect of electrons on the second carbon of the pair is established and real kationoid (acceptor) activity becomes

[78] Robinson, *J. Soc. Dyers Colourists, Jubilee Issue*, 65 (1934).

possible." The contribution of external acceptor and donor centers in the activation of olefins and carbonyl compounds may be expected to vary with the environment; in either case the seat of unsaturation in the activated form must be the incomplete valence shell of the deficient atom.

In systems containing covalent triple bonds the theoretical considerations are essentially the same as those applied to the olefins. The addition reactions of acetylenes and of nitriles indicate that the dominant active forms are those anticipated.

$$R\text{---}\overset{\frown}{C}\text{≡}CH + HOH \xrightarrow{H_2SO_4} R\text{---}C(OH)\text{=}CH_2 \rightarrow R\text{---}CO\text{---}CH_3$$

$$R\text{---}C\text{≡}C\text{---}H + H\text{---}OCH_3 \xrightarrow{BF_3} R\text{---}C(OCH_3)\text{=}CH_2 \rightarrow R\text{---}C(OCH_3)_2\text{---}CH_3$$

$$R\text{---}C\text{≡}CH + HBr \longrightarrow R\text{---}CBr\text{=}CH_2$$

$$R\text{---}\overset{\frown}{C}\text{≡}N + HOH \longrightarrow R\text{---}C(OH)\text{=}NH \rightarrow R\text{---}CO\text{---}NH_2$$

$$R\text{---}C\text{≡}N + HX \longrightarrow R\text{---}CX\text{=}NH$$

$$R\text{---}C\text{≡}N + R\text{---}MgX \longrightarrow R_2C\text{=}N\text{---}MgX$$
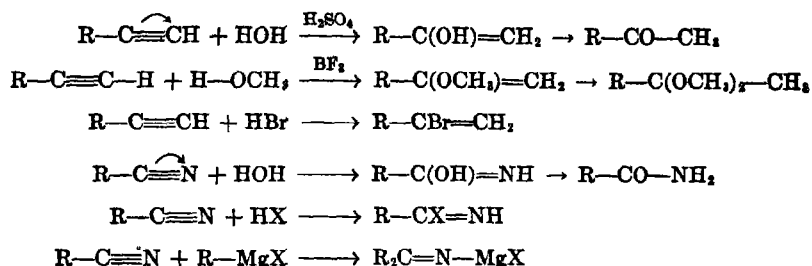
The diazonium cations and acetylide anions, $[R\text{---}N\text{≡}N]^+$ and $[R\text{---}\underset{\alpha}{C}\text{≡}\underset{\beta}{C}\text{---}]^-$, afford examples of the effect of ionic charges upon the configuration of the active forms. In the diazonium cations the large residual positive charge of the 4-covalent nitrogen tends to bring about withdrawal of an electron pair from the multiple link and to create an electron deficit on the β-nitrogen. Under appropriate conditions an active donor may add at this seat of unsaturation and give rise to diazo structures (a). On the other hand, under certain conditions unstable

(a) $[R\text{---}\overset{\frown}{N}\text{≡}N]^+ + [:OH]^- \rightarrow R\text{---}N\text{=}N\text{---}OH$

(b) $[R\text{--→}N\text{≡}N]^+ Cl^- + CuCl \rightarrow [R\text{--→}N_2\text{---}CuCl_2] \rightarrow R\text{---}Cl + N_2 + CuCl$

(c) $[R\text{---}\overset{\frown}{C}\text{≡}C\text{---}]^- + 2H\text{---}OC_2H_5 \rightarrow R\text{---}CH\text{=}CH\text{---}OC_2H_5 + [C_2H_5O]^-$
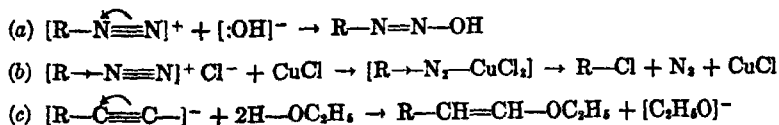
complexes can be formed in which the strong electron-attraction of the positively charged center will facilitate migration of an organic cation (b).

In the acetylide anions the high electron density of the terminal carbon will favor electron-release, and the direction of the electromeric effect may be expected to be the reverse of that in the acetylene itself. Indeed, the orientation of the addition of alcohols to acetylenes is reversed in the presence of strong alkalies (c).

The covalent triple bonds in carbon monoxide and the isocyanides are of an unusual type, owing to the presence of an unshared electron

pair on the carbon atom in the normal state of the molecule.[77] The strong electron-attraction of oxygen or nitrogen will tend to create an electron-deficit on the carbon atom and facilitate its coördination with a donor center. In the resulting complex an acceptor center may then migrate to the unshared electron pair of the carbon (α,β-shift), and the net result is that both fragments of the addendum become attached to the carbon atom.

$$:C\!\!\equiv\!\!O: + Cl_2 \rightarrow \left[Cl\!-\!Cl\!\rightarrow\!C\!\!=\!\!\ddot{O}: \right] \xrightarrow[\text{shift}]{\alpha,\beta\text{-}} Cl\!-\!C\!\!=\!\!O$$
$$\underset{\delta-\;\;\;\delta+}{} \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\underset{\displaystyle Cl}{\big|}$$

$$:C\!\!\equiv\!\!O: + [OH]^- \rightarrow \left[H\!-\!O\!-\!C\!\!=\!\!\ddot{O}: \right]^- \rightarrow \left[\begin{array}{c} O\!-\!C\!\!=\!\!O \\ \big| \\ H \end{array} \right]^-$$

$$:C\!\!\equiv\!\!N\!-\!R + H\!-\!OH \rightarrow \left[\begin{array}{c} H\!-\!O\!-\!C\!\!\equiv\!\!\ddot{N}\!-\!R \\ \big| \\ H \end{array} \right] \rightarrow \begin{array}{c} H\!-\!O\!-\!C\!\!=\!\!N\!-\!R \\ \big| \\ H \end{array}$$
$$\updownarrow \;\; \alpha,\gamma\text{-shift}$$
$$O\!\!=\!\!CH\!-\!NH\!-\!R$$
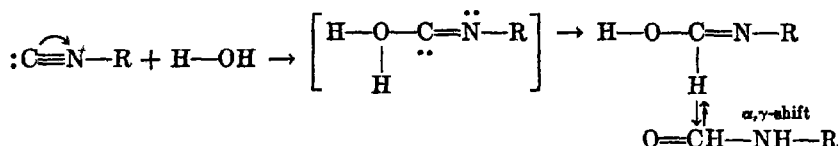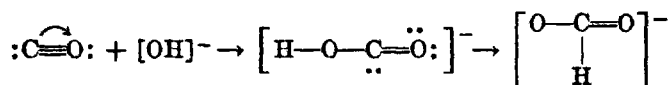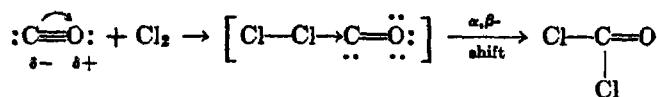
The physical properties and reactions of these systems indicate that carbon atoms which have been represented as bivalent in the conventional formulas actually have a complete valence octet made up of a triple covalent link and an unshared electron pair.

POLYFUNCTIONAL ELECTROMERIC SYSTEMS

The union of two or more centers that are capable of taking part in electromeric displacements may give rise to systems of diminished reactivity owing to internal compensation (resonance effects), or of enhanced reactivity resulting from a favorable combination of activating effects. The direct attachment of one or more unsaturated units, such as —CH=CH— or —C≡C—, to an active donor or acceptor center permits a transmission of the activity to a more remote atom in the system. Thus, the acceptor activity of the carbon atom of a carbonyl group or olefin may be transmitted to the β-atom of an attached unsaturated system; the donor activity of oxygen or nitrogen may be transferred in a

$$O\!\!=\!\!CH\!-\!CH\!\!=\!\!CH_2 \qquad\qquad CH_2\!\!=\!\!CH\!-\!CH\!\!=\!\!CH\!-\!R$$
$$\alpha\quad\;\;\beta$$

$$HO\!-\!CH\!\!=\!\!CH\!-\!R \qquad\qquad H_2N\!-\!CH\!\!=\!\!CH\!-\!R$$
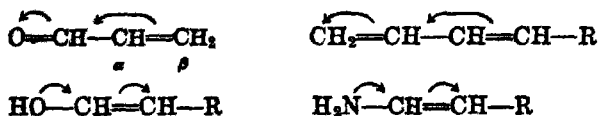
[77] Hammick, New, Sidgwick, and Sutton, *J. Chem. Soc.*, 1876 (1930); Sidgwick, *Chem. Rev.*, **9**, 77 (1931).

similar way to the β-carbon in an attached group. The 1,4-addition reactions of α,β-unsaturated carbonyl compounds and of 1,3-dienes are familiar examples of the first type; the carbon alkylation of ethyl acetoacetate and β-aminocrotonate illustrate the second type.

Vinylogous Systems. Fuson [78] has formulated the following generalization concerning the propagation of the influence of a functional group along an unsaturated chain: When, in a system of the type X—Y=Z or X—Y≡Z, a structural unit of the type $-(C=C)_n-$ is interposed between X and Y, the function of Z remains qualitatively unchanged but that of Y may be usurped by the carbon atom attached to X. It is proposed to term such a group of compounds a vinylogous series, and the members of the series vinylogs of one another. Thus, acetaldehyde, crotonaldehyde, and sorbic aldehyde may be regarded as a vinylogous series, where X is CH_3, Y=Z is —CH=O, and $n = 0$,

$$CH_3—CH=O \qquad CH_3—CH=CH—CH=O \qquad CH_3—CH=CH—CH=CH—CH=O$$
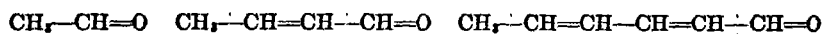
1, and 2. Evidence of the effect of the carbonyl group on the terminal methyl groups in crotonaldehyde and sorbic aldehyde is shown by their ability to undergo aldol condensations forming the higher members of the series. Likewise, ethyl crotonate and sorbate undergo condensation with ethyl oxalate in a manner similar to the reaction of ethyl acetate.

$$CH_3—(CH=CH)_n—CO_2Et + \overset{\displaystyle CO_2Et}{\underset{\displaystyle CO_2Et}{|}} \rightarrow \overset{\displaystyle CO—CH_2—(CH=CH)_n—CO_2Et}{\underset{\displaystyle CO_2Et}{|}}$$
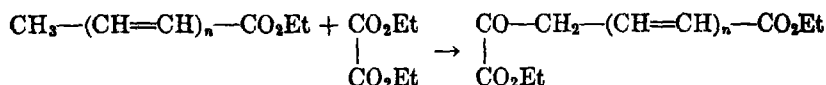
In disubstituted aromatic derivatives of the type A—C_6H_4—B, the *ortho* and *para* compounds will have a vinylogous relationship to the system A—B, but the *meta* isomer will not be a vinylog of A—B. Thus, the methyl group of *o-* and *p-*nitrotoluene is activated, resembling $CH_3—NO_2$, but that of *m-*nitrotoluene is not.

$$CH_3—NO_2 \qquad CH_3—C \overset{\displaystyle \diagup}{=} C—NO_2 \qquad CH_3—C=CH—CH=C—NO_2$$
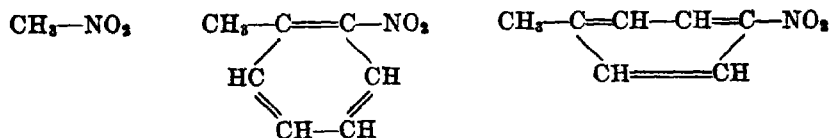
Hetero-Enoid Systems. The combination of a hetero atom such as nitrogen, oxygen, or sulfur (in their lower covalent states) with an unsaturated system by means of a single link is termed by Robinson a hetero-enoid system. In these structures the hetero atom tends to increase its covalence with the α-carbon of the unsaturated system, and

[78] Fuson, *Chem. Rev.*, **16**, 1 (1935); see, also, Chapter 7, p. 633.

as a result the donor activity (seat of attack for acceptor reagents) may be transmitted to the β-carbon atom.

$$\text{H}_2\text{N}\!-\!\text{C}\!=\!\text{C} + \text{CH}_3\!-\!\text{X} \rightarrow [\text{H}_2\text{N}\!=\!\text{C}\!-\!\text{C}\!-\!\text{CH}_3]^+\text{X}^-$$
$$\quad\;\; \alpha \;\;\; \beta \qquad\qquad\qquad\qquad \alpha \;\;\;\; \beta$$
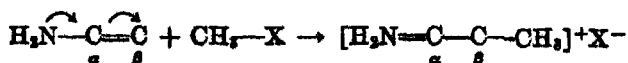
Activation of the *o*- and *p*- positions of the aromatic ring toward acceptor reagents, by substituents such as —OH, —OR, —NH$_2$, and the halogens, may be attributed to the transmission of the donor activity of these hetero atoms.

The effectiveness of the hetero atoms in increasing the degree of polarization of an attached ethylenic or aromatic system involves the interplay of several distinct polar effects, and the observed sequence will vary according to the relative contribution of each effect in the reaction or in the equilibrium under consideration. As a practical guide Robinson gives the following rule: if a series of bases X—H, X'—H, X''—H is arranged in the order of diminishing proton affinity, then the systems X—C=C, X'—C=C, X''—C=C are arranged in the order of *diminishing polarization and of diminishing donor reactivity exhibited* by the carbon atoms. Negatively charged groups as in the phenoxide and enol anions will occur at the top of the scale of effectiveness, but the participation of the hetero atom in some other conjugated electromeric system (involving its available electrons) will diminish its effectiveness. The order of effectiveness will therefore be:

$$\text{—}\overset{-}{\text{N}}\text{H} > \text{—}\overset{-}{\text{O}} > \text{—NH}_2 > \text{—OH} > \text{—O—CO—CH}_3 > \text{—I} > \text{—Cl}$$

$$\text{—NHCH}_3 > \text{—NH}_2 > \text{—NH—C}_6\text{H}_5 > \text{—NH—CO—R} > \text{—N(CO—R)}_2$$

$$\text{—S—CH}_3 > \text{—O—CH}_3 > \text{—O—C}_6\text{H}_5 > \text{—O—CO—R} > \text{—(SR}_2)^+$$
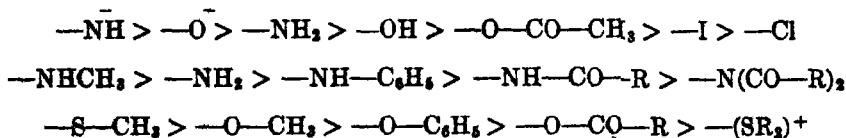
The heterocycles of aromatic type (p. 127) such as pyridine, thiophene, furan, and pyrrole may be regarded as hetero-enoid cycles.

Neutralized Systems. The union of a donor and acceptor center may be expected to result in diminished reactivity of the system owing to internal compensation. This effect is evident in the union of the carbonyl group with donor systems such as —OH and —NH$_2$. In a series of systems of the type X—C=Y, when Y is kept constant, the strength of the internal effect will depend upon the electron-release of X, and will correspond to the following sequence:

$$\overset{-}{\text{O}}\!-\!\text{C}\!=\!\text{O} > \text{R}_2\text{N}\!-\!\text{C}\!=\!\text{O} > \text{RO}\!-\!\text{C}\!=\!\text{O} > \text{Cl}\!-\!\text{C}\!=\!\text{O} \gg \text{H}\!-\!\text{C}\!=\!\text{O}$$
$$\quad\;\; | \qquad\qquad | \qquad\qquad | \qquad\qquad | \qquad\qquad\; |$$
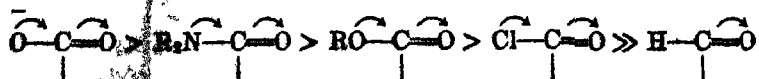
In the carboxylate ion the transference is equivalent to half an electron,

since the mesomeric form of the anion is symmetrical with respect to the oxygen atoms.

The transition for the carboxylate anion involves merely the *displacement* of an ionic center, but that for formally neutral systems (amides, esters, acyl halides) requires the *creation* of an electrical dipole. The mesomeric effect is therefore much less than that required for a completed interchange. Physical evidence indicates that the order of magnitude of the mesomeric polarization of formally neutral systems is actually about one-tenth of that required for complete polarization. The effect of the whole group $X\!-\!C\!=\!Y$ upon a system attached at C and the neutralization effect within the group are opposed to each other. Consequently the permanent positive polarization (and the acceptor activity) of the carbon atoms in these systems is the reverse of the above sequence.

If X remains constant and Y is varied, the extent of internal neutralization will be determined by the $+T$ effect of Y. This may be illustrated by the following series:

$$R_2N\!-\!\overset{+}{C}\!=\!NR_2 > R_2N\!-\!C\!=\!O > R_2N\!-\!C\!=\!NR$$
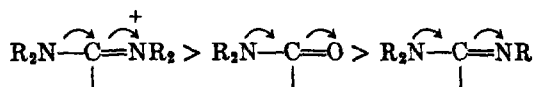
In this instance the acceptor activity of the carbon atom follows the same sequence and is not reversed, since the effect of R_2N- remains constant.

The effect of variations in the central atom may be shown by a comparison of structures represented by the general formula $X\!=\!Y\!-\!X$, where X is oxygen and Y is carbon, nitrogen, sulfur, or oxygen. In this series the acceptor activity of the central atom increases as its effective nuclear charge becomes greater.

$$\overset{+}{O\!=\!O}\!-\!\bar{O} > \overset{+}{O\!=\!S}\!-\!\bar{O} > \underset{R}{\overset{+}{O\!=\!N}}\!-\!\bar{O} > \underset{R}{O\!=\!C}\!-\!\bar{O}$$
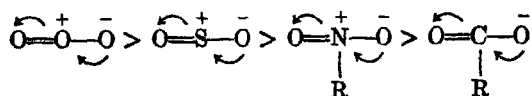
In other neutralized systems of the form $X\!=\!Y\!-\!Z$, the general principles outlined above may be used to judge qualitatively the relative reactivity of a series of related compounds.

1,2-Dienoid Systems. The 1,2-dienes (allenes) and related compounds containing doubly linked nitrogen and oxygen may be written in the general form $X\!=\!Y\!=\!Z$. These systems may be considered from the standpoint of the availability of the electron pairs of the multiple links and of unshared electron pairs on the atoms X and Z. The trend of the electron displacements may be considered conveniently by com-

paring types in which one or two of the components of the systems remain constant. In the first series indicated below, where X=Y is $R_2C=C$, the polarization of Z (and electron deficit of Y) increases from the allenes to the ketenes. The internal effect of neutralization due to a compensating mesomeric electron-release by X must be extremely small since X does not have an unshared electron pair.

$$R_2C=C=CH_2 \qquad R_2C=C=O \qquad R_2C=C=CR_2$$

$$R_2C=C=NR \qquad R—N=C=O \qquad R—N=C=N—R$$

$$R_2C=C=O \qquad R—N=N=CR_2 \qquad O=C=O$$

In the principal active form the atom Z withdraws an electron pair of the link Y=Z, giving rise thereby to an acceptor center at Y and a donor center at Z. The reactivity of these systems toward donor reagents will increase as the effective nuclear charge of Z becomes greater.

In their reactions with unsymmetrical reagents the donor center of the reagent will become attached at Y but the acceptor center of the reagent may become linked at either X or Z, owing to the intervention of electromeric shifts in the course of reaction.

$$\overset{3}{R_2}C=\overset{2}{C}=\overset{1}{CH_2} + \overset{\delta-}{A}—B \rightarrow \left[\begin{array}{c} R_2C=C—CH_2— \\ \uparrow \\ A—B \end{array} \right] \rightarrow \begin{array}{c} R_2C=C—CH_2—B \\ | \\ A \\ \text{1,2-addition} \end{array}$$

$$\Updownarrow$$

$$\begin{array}{c} R_2C=C—CH_2 \\ | \\ A—B \end{array} \rightarrow \begin{array}{c} R_2C—C=CH_2 \\ |\ \ \ | \\ B\ \ A \\ \text{2,3-addition} \end{array}$$

The fact that the allenes of type $R_2C=C=CH_2$ yield exclusively methyl ketones upon hydration indicates a marked selectivity in the point of attack of the donor center of the reagent. The addition of hydrogen bromide leads to a mixture of isomeric 2-bromo olefins which may arise by fixation of the acceptor center of the reagent in the 1- or 3-positions.[79] The absence of corresponding isomers in the hydration reaction may be attributed to the fact that 1,2- or 2,3-addition of water would merely give isomeric enol forms of the methyl ketone.

$$R_2C=C(OH)—CH_3 \text{ and } R_2CH—C(OH)=CH_2$$

Ingold has pointed out that a number of systems of the general form X=Y=Z contain systems capable of a mesomeric state owing

79 Bouis, Ann. chim., [10] 9, 402 (1928).

to the presence of an unshared electron pair on the atom X or Z. The alternative structure in this case would contain a triple link and would have the general form X—Y≡Z. The second series given above includes the ketenes, isocyanates, and aliphatic diazo compounds as characteristic examples. The ketenes and aliphatic diazo compounds illustrate systems in which only one of the terminal atoms can furnish an unshared electron pair. In the azides and isocyanates either terminal atom can furnish an unshared pair, and two different mesomeric polarizations are possible (Table XIX).

TABLE XIX

MESOMERIC EFFECTS IN STRUCTURES X=Y=Z

Type	Formula	Mesomeric Polarization	Alternative Structure
Ketenes	$R_2C=C=O$	$R_2C=C=O$	$R_2\ddot{C}—C≡O$
Diazo compounds	$R_2C=N=N$	$R_2C=N=N$	$R_2\ddot{C}—N≡N$
Azides	$R—N=N=N$	$R—N=N=N$ (major)	$R—\ddot{N}—N≡N$
		$R—N=N=N$ (minor)	$R—N≡N—\ddot{N}$
Isocyanates	$R—N=C=O$	$R—N=C=O$ (major)	$R—N≡C—\ddot{O}$
		$R—N=C=O$ (minor)	$R—\ddot{N}—C≡O$
Carbon dioxide	$O=C=O$	$O=C=O$	$\ddot{O}—C≡O$

The addition of alcohols and amines to ketenes and isocyanates may be regarded as a direct combination of the donor center of the addendum at the carbonyl carbon. The postulation of an enol form of the intermediate is unnecessary since the acceptor center of the addendum may shift directly to give the more stable form (ester or amide).

$$R_2C=C=O \xrightarrow{:NH_3} R_2C=C-O \longrightarrow R_2CH-C=O$$
$$\qquad\qquad\qquad\qquad\quad | \qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad NH_3 \qquad\qquad\qquad\qquad NH_2$$
$$\qquad\qquad\qquad\qquad | \nearrow$$
$$\qquad\qquad\qquad R_2C=C-OH$$
$$\qquad\qquad\qquad\qquad |$$
$$\qquad\qquad\qquad\qquad NH_2$$
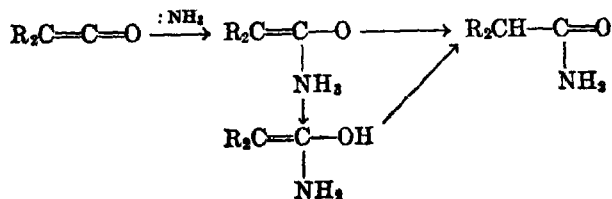
The addition of halogen acids may occur by a similar mechanism or may be initiated by coördination of the carbonyl oxygen with an acceptor center of the addendum. The fact that ketenes generally react more rapidly with active donor addenda than with acceptors may be considered to favor the former view.

The reaction of ketenes and isocyanates with Grignard reagents has been studied by Gilman,[30] and in these cases there is undoubtedly a preliminary coördination of the carbonyl oxygen.

$$R_2C=C=O \rightarrow Mg\begin{matrix} X \\ R \end{matrix} \rightarrow R_2C=C-OMgX \rightarrow R_2C=C\begin{matrix} OH \\ R \end{matrix}$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\quad R$$
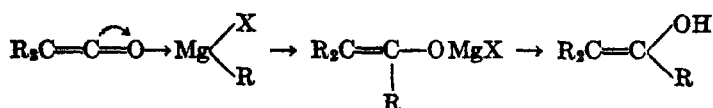
Diphenylketene, on treatment with phenylmagnesium bromide and subsequent hydrolysis, was found to give the stable enol form of diphenylacetophenone (pp. 514 and 663). A similar mechanism was demonstrated experimentally for the isothiocyanates.

Although the existence of alternative structures of the type $R_2\overset{..}{C}-C\equiv O$ (Table XIX) for the ketenes has not been verified experimentally, the fact that acyl substituted ketenes show a strongly diminished acceptor activity suggests an internal neutralization of the following type:

$$O=C-CH=C=O \qquad\qquad \overset{..}{O}-C=CH-C\equiv\overset{+}{O}$$
$$\quad | \qquad\qquad\qquad\qquad\qquad\qquad | $$
$$\quad R \qquad\qquad\qquad\qquad\qquad\qquad R$$
$$\quad\ \ I \qquad\qquad\qquad\qquad\qquad\qquad\quad II$$

In these structures the strong $+E$ effect of the α,β-unsaturated carbonyl system opposes the normal electromeric polarization of the ketene carbonyl group and reinforces the mesomeric polarization. In the resulting structure (II) the electronic excess is transmitted to the carbonyl oxygen and the resonance between I and II has the effect of diminishing the reactivity toward an external donor molecule.

1,3-Dienoid and Polyenoid Systems. The union of two or more multiple bonds in a 1,3- or 1,3,5-relationship gives rise to an interaction (conjugation) within the system. The extent of this interaction varies over a wide range, and the behavior of the systems is influenced by internal and external factors. Conjugate addition at the 1,4- or 1,6-positions arises from the ability of the system to transmit an electronic deficit, resulting from the electromeric polarization of one multiple

bond, to the terminal atom of the second or third multiple bond (a and b).

(a) —C=C—C=C—
1 2 3 4

(b) —C=C—C=C—C=C—
1 2 3 4 5 6
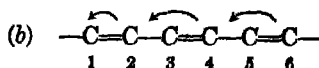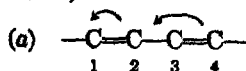
Aside from the conjugate active form the 1,3-diene may exist in non-conjugate active forms (a', a'') and the 1,3,5-triene in partially conjugate or non-conjugate active forms (b', b'').

(a') —C=C←—C=C—

(b') —C=C—C=C←—C=C—
1 2 3 4   5 6

(a'') —C=C—→C=C—

(b'') —C=C←—C=C←—C=C—
1 2   3 4   5 6
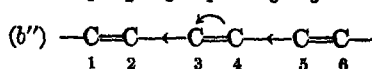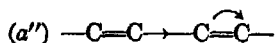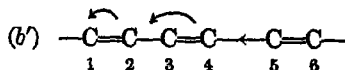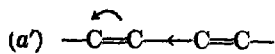
It has sometimes been assumed [81] that typical 1,4-addition reactions of the dienes involve a completed 1,2-addition followed by allylic rearrangement. The interpretation of much of the experimental evidence bearing upon this point is uncertain owing to the facile interconversion of the isomeric 1,2- and 1,4-adducts, and the effects of oxygen and peroxides.[82] Nevertheless, there is now definite evidence that chlorine [83] and halogen acids [30] yield 1,4-adducts directly in certain instances, and the hypothesis that they necessarily arise by allylic rearrangement must be abandoned. Reagents such as halogens and halogen acids usually produce a mixture of 1,2- and 1,4-adducts, whereas perbenzoic acid [84] yields only 1,2-adducts (substituted ethylene oxides), and maleic anhydride only 1,4-adducts [85] (p. 668).

Kharasch and his collaborators [82] have found that, in the absence of oxygen and peroxides, and in the presence of an antioxidant, butadiene adds hydrogen bromide at low temperatures to give principally the 1,2-adduct, 3-bromo-1-butene (II). At higher temperatures under the influence of hydrogen bromide, and particularly under the combined influence of hydrogen bromide and peroxides, this product rearranges to I. The addition product obtained in the presence of air or added peroxides is principally I (crotyl bromide). Evidence is lacking to show whether peroxides cause direct formation of crotyl bromide by 1,4-addition of hydrogen bromide to butadiene, or merely rearrange the 1,2-addition product. All that can be said with assurance is that in

[81] Claisen and collaborators, J. prakt. Chem., 105, 74 (1922); Gillet, Bull. soc. chim. Belg., 31, 366 (1922); Ingold, Shoppee, and Thorpe, J. Chem. Soc., 1477 (1926); Burton, ibid., 1651 (1928); Farmer and Scott, ibid., 172 (1929).

[82] Kharasch, Margolis, and Mayo, J. Org. Chem., 1, 393 (1936).

[83] Muskat and Northrup, J. Am. Chem. Soc., 52, 4043 (1930).

[84] Pummerer and Reindel, Ber., 66, 335 (1933).

[85] Diels and Alder, Ann., 460, 98 (1928); 478, 139 (1930).

the absence of peroxides 1,4-addition does not occur *with hydrogen bromide*. A careful but unsuccessful search was made for the third possible addition product, III. Two independent analytical methods indicate that not more than 5 per cent of this substance could have been present in the reaction products.

$$
\begin{array}{l}
\text{H H H H} \\
\text{HC} \!=\! \text{C} \!-\! \text{C} \!=\! \text{CH} + \text{HBr}
\end{array}
\left\{
\begin{array}{ll}
\text{CH}_3\text{—CH}\!=\!\text{CH—CH}_2\text{Br} & \text{(1-bromo-2-butene)} \quad \text{I} \\[4pt]
\text{CH}_2\!=\!\text{CH—CH—CH}_3 \\
\qquad\qquad\;\; | \\
\qquad\qquad\; \text{Br} & \text{(3-bromo-1-butene)} \quad \text{II} \\[4pt]
\text{CH}_2\!=\!\text{CH—CH}_2\text{—CH}_2\text{Br} & \text{(4-bromo-1-butene)} \quad \text{III}
\end{array}
\right.
$$

<center>Possible reactions of butadiene and hydrogen bromide</center>
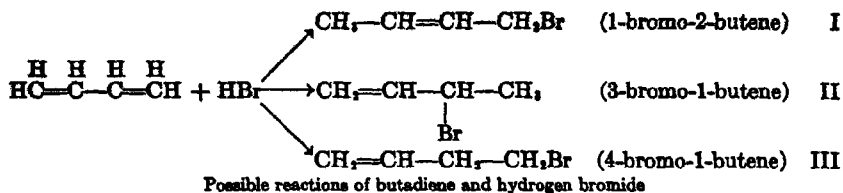
Considerations of the 1,2- and 1,4-addition of hydrogen and of bromine to butadiene, based upon the quantum mechanics, indicate that it is much easier for addition to take place in the 1,4-positions.[96] This conclusion rests upon the observation that calculated values of the activation energy for the 1,4-reactions are appreciably smaller than those for the 1,2-reactions. The introduction of a substituent may alter the situation and make 1,2-reaction occur more readily.

The degree of electromeric polarization of the 1,3-dienes must be very small or spontaneous polymerization would take place even in the absence of catalysts. Probably effective polarization occurs through the intervention of an active center of the reagent or catalyst. Unsymmetrical substitution will generally enhance the small permanent polarization and will exert a directive effect. In 2-methylbutadiene (isoprene) the $-I$ effect of the alkyl group will favor the development of donor activity at the 1-position, whereas in 1-bromobutadiene the substituent will favor the development of donor activity at the remote position.

$$
\begin{array}{l}
\qquad\; \text{CH}_3 \\
\qquad\;\; | \\
\text{CH}_2\!=\!\text{C—CH}\!=\!\text{CH}_2 \qquad\qquad\qquad \text{Br—CH}\!=\!\text{CH—CH}\!=\!\text{CH}_2
\end{array}
$$
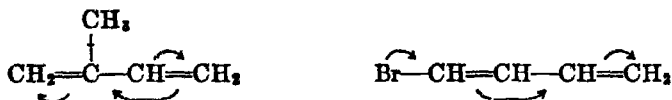
The occurrence of this orientation in isoprene is confirmed by the fact that conjugate addition of hydrogen bromide yields 2-methyl-4-bromobutene-2, and that non-conjugate addition reactions yield 1,2- rather than 3,4-adducts.

Robinson has suggested that the polymerization of 1,3-dienes under the influence of a trace of sodium is a chain reaction initiated by the effective polarization of one molecule of the diene resulting from the entry of an electron or electron pair at the acceptor center; the polarized molecule attacks a second molecule of the diene in the same fashion, and

[96] Eyring, Sherman, and Kimball, *J. Chem. Phys.*, **1**, 586 (1933).

the process continues until long chains or large rings are formed (p. 1932). Molecular oxygen and peroxides may play a similar role in the initiation of polymerization reactions. It is possible that a chain reaction of opposite type may be initiated by the intervention of an external acceptor, since the diene system is capable of functioning either as a donor or an acceptor.

Donor → CH₂=CH—CH=CH₂ CH₂=CH—CH=CH₂ etc.

Acceptor CH₂=CH—CH=CH₂ CH₂=CH—CH=CH₂←etc.
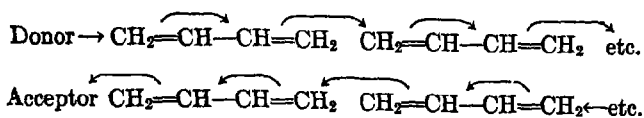
The aliphatic 1,3-dienes generally show a reactivity greater than that of the simple olefins toward the usual reagents, such as halogens and halogen acids, and they undergo a number of reactions that are not shown by compounds having an isolated double bond. The characteristic diene reactions include coupling with nitrobenzenediazonium chloride,[87] facile linear polymerization under the influence of alkali metals, direct addition of alkali metals [88] and of triphenylmethyl,[89] and the Diels-Alder reaction with α,β-unsaturated carbonyl compounds [85] (p. 685).

Thermochemical data show that the heat of formation of a conjugated system is greater than the sum of the heats of formation of the separate bonds. This departure from strict additivity corresponds to an increase in molecular stability and is interpreted as resonance energy (perturbation energy). Quantum-mechanical calculations [90] have led to values of resonance energies that can be brought into good agreement with those obtained from heats of combustion. The numerical values for several types of conjugated systems, expressed in kilocalories per mole, are tabulated below. Numbers in italics are calculated values; the remainder are empirical values from thermochemical data.

TABLE XX

RESONANCE ENERGIES OF CONJUGATED SYSTEMS
(PAULING AND SHERMAN)

1,3-Dienes	*8.0*	Benzene	*37.3*	Pyridine	43.1
1,3,5-Trienes	*16.7*	Styrene	46 1	Pyrrole	22.6
1,3,5,7-Tetrenes	*25.1*	Stilbene	94.3	Thiophene	31.1
Fulvenes	*15.0*	Naphthalene	74.7	Furan	21.4

[87] Meyer and collaborators, Ber., 47, 1754 (1914); 52, 1472 (1919).
[88] Ziegler, Orth, and Weber, Ann., 479, 292 (1930); 504, 131 (1933).
[89] Conant and Scherp, J. Am. Chem. Soc., 53, 1941 (1931).
[90] Pauling and Sherman, J. Chem. Phys., 1, 606, 679 (1933).

The marked effect of conjugation upon the energetics of unsaturated systems is demonstrated by a comparison of the heats of hydrogenation of olefins, dienes, and benzene.[51] When two double bonds are separated by three single bonds, as in 1,5-hexadiene, there is practically no interaction; the heat of hydrogenation is twice that of a simple olefin of corresponding type. There appears to be a small labilizing effect in the 1,4-diene, and in the 1,2-diene a large labilizing effect. The 1,3-diene instead of exhibiting a labilizing effect of intermediate magnitude actually shows a definite stabilizing effect. Similar effects occur in cyclopentadiene and cyclohexadiene, and an enormous effect arises in benzene. Table XXI gives the heats of hydrogenation (ΔH, in kilocalories per mole) for several olefins and polyenes, together with the magnitude of the effects of stabilization ($+\Delta Z$) or labilization ($-\Delta Z$).

<div align="center">

TABLE XXI

HEATS OF HYDROGENATION OF OLEFINS AND POLYENES

</div>

Olefins	$-\Delta H$	Polyenes	$-\Delta H$	ΔZ
$CH_2\!=\!CH_2$	32.82	1,2-Propadiene	71.28	-14.5^*
R—CH$=$CH$_2$ (mean)	30.20	1,3-Butadiene	57.07	$+ 3.3$†
CH$_3$—CH$=$CH—CH$_3$ *cis*	28.57	1,4-Pentadiene	60.79	$- 0.4$†
trans	27.62	1,5-Hexadiene	60.52	$- 0.1$†
(CH$_3$)$_2$C$=$CH$_2$	28.37	Cyclopentadiene	50.86	$+ 6.3$‡
(CH$_3$)$_2$C$=$CH—CH$_3$	26.92	Cyclohexadiene	55.37	$+ 1.8$‡
Cyclohexene	28.59	Benzene	49.80	$+36.0$‡

<div align="center">

* Referred to isobutylene.
† Referred to R—CH $=$ CH$_2$.
‡ Referred to cyclohexene.

</div>

The calculated resonance energy of benzene (Table XX) is in good agreement with the stabilization effect observed by this method, but the calculated value for 1,3-dienes shows a wider deviation from the actual values.

In benzene and similar systems of aromatic character the internal compensation has the effect of facilitating substitution rather than simple addition reactions. However, there is evidence to support the view that substitution reactions of a number of aromatic systems proceed by way of a preliminary addition process (p. 174). Halogen atoms, hydroxyl, and amino groups directly attached to an aromatic ring give rise to hetero-enoid systems in which the electromeric effect

[51] Kistiakowsky, Ruhoff, Smith, and Vaughan, *J. Am. Chem. Soc.*, **57**, 876 (1935); **58**, 146 (1936); Conant and Kistiakowsky, *Chem. Rev.*, **20**, 181 (1937).

$(-E)$ of the substituent interacts with the polyenoid system (see hetero-enoid systems and vinylogous systems). It is possible that a part of the difficulty encountered in developing a satisfactory general theory of aromatic substitution is due to the situation that the reactions can occur either by a direct or an indirect (addition) mechanism.

Triple bonds are capable of taking an active part in conjugated systems (p. 667). The combination of a double and triple bond, as in vinylacetylene, gives rise to a system that undergoes only conjugate addition with halogen acids.[92] In this instance the orientation of the addition is determined by the triple bond. Divinylacetylene likewise adds chlorine or hydrogen chloride in the 1,4-positions;[93] no evidence of 1,6-addition to divinylacetylene has been observed.

$$CH_2{=}CH{-}C{\equiv}CH + HCl \rightarrow Cl{-}CH_2{-}CH{=}C{=}CH_2$$

$$CH_2{=}CH{-}C{\equiv}C{-}CH{=}CH_2 + HCl \rightarrow Cl{-}CH_2{-}CH{=}C{=}CH{-}CH{=}CH_2$$

$$CH_2{=}CH{-}C{\equiv}C{-}CH{=}CH_2 + Cl_2 \rightarrow Cl{-}CH_2{-}CH{=}C{=}CCl{-}CH{=}CH_2$$

$$R{-}C{\equiv}C{-}C{\equiv}C{-}R + H_2 \rightarrow R{-}CH{=}C{=}C{=}CH{-}R + R{-}CH_2{-}C{\equiv}C{-}CH_2{-}R$$

$$R{-}C{\equiv}C{-}C{\equiv}C{-}R + H_2O \rightarrow (R{-}C{=}C{=}C{=}CH{-}R)$$
$$\overset{|}{O}H \qquad \rightarrow R{-}CO{-}C{\equiv}C{-}CH_2{-}R$$
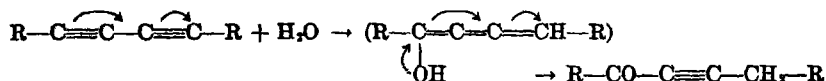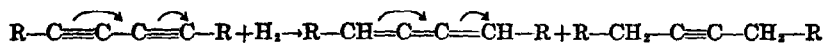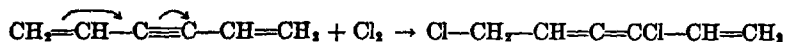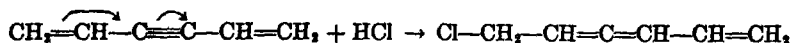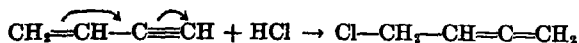
Studies of substituted 1,3-diynes by Grignard and Tcheoufaki[94] demonstrate that systems containing two triple bonds undergo conjugate addition of bromine and hydrogen bromide. Similarly, catalytic hydrogenation over platinum, and partial hydration, lead to 1,4-adducts.

α,β-Unsaturated Carbonyl Systems and Related Types.* The conjugation of an ethylenic linkage with an unsymmetrical multiple bond of oxygen or nitrogen gives rise to structures of the type $C{=}C{-}C{=}A$ and $C{=}C{-}B{=}A$. In the single links of oxygen and nitrogen (heteroenoid systems) the hetero atom exerts a dynamic electron-release, but in their multiple bonds the direction of the electromeric effect is reversed.

$$C{=}C{-}OR \qquad\qquad C{=}C{-}C{=}O$$

$$C{=}C{-}NR_2 \qquad\qquad C{=}C{-}C{\equiv}N$$
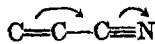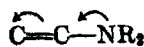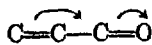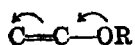
Hetero-enoid   Katio-enoid

[92] Carothers, Berchet, and Collins, *J. Am. Chem. Soc.*, **54**, 4066 (1932).

[93] Coffman and Carothers, *ibid.*, **55**, 2040, 2048 (1933).

[94] Grignard and Tcheoufaki, *Compt. rend.*, **188**, 527, 1531 (1929).

* These were classified by Robinson originally as "crotonoid" and later as "katio-enoid" systems.

The essential feature of the α,β-unsaturated carbonyl types (p. 672) is the ability of the system to transfer the seat of acceptor activity to the β-carbon of the ethylenic bond. Consequently, the β-carbon is attacked by the donor center of various reagents, such as ammonia, amines, alcohols, organomagnesium halides, and alkali cyanides, which are without action on simple olefins.

The addition of amines, alcohols, and alkali cyanides to α,β-unsaturated carbonyl systems may be regarded as a direct combination of the donor center of the addendum at the β-carbon, but in the addition of Grignard reagents and halogen acids it is likely that the reaction is initiated by coördination of the hetero atom with an acceptor center of the addendum. The tendency to permanent polarization in the α,β-unsaturated carbonyl systems is necessarily small, owing to the fact

$$H_2N: + C{=}C{-}C{=}O \rightarrow H_2\overset{+}{N}{-}C{-}C{=}C{-}\overset{-}{O}: \rightarrow H_2N{-}C{-}CH{-}C{=}O$$
$$\quad\quad\quad \beta \;\; \alpha \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad \beta \;\; \alpha$$

$$C{=}C{-}C{=}O + HCl \rightarrow \left[C{=}C{-}C{=}OH\right]^{+}\overset{-}{Cl} \rightarrow Cl{-}C{-}C{=}C{-}OH$$
$$\beta \;\; \alpha \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad \beta \;\; \alpha$$
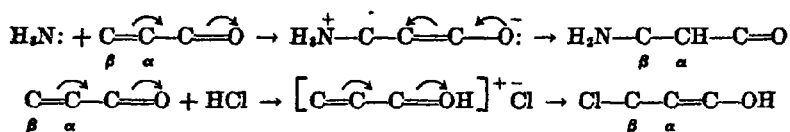
that integral polarization produces an unstable electronic configuration (open-sextet) in the β-position.

Although α,β-unsaturated carbonyl systems show a marked tendency to undergo conjugate addition, there are many instances in which one of the multiple bonds functions independently. The relative amounts of 1,2- and 1,4-adducts are influenced by substituents in the conjugated system (internal factors) and also by the nature of the addendum and the environment (external factors). Kohler [48] has pointed out that nearly all reactions involving 1,2-addition to carbonyl are reversible, whereas the products formed by 1,4-addition (except with organometallic compounds) undergo rearrangement into saturated carbonyl compounds that are still capable of undergoing 1,2-addition. Under these conditions the products ultimately isolated do not represent the relative rates of 1,2- and 1,4-addition but merely the relative stability of the substances or the position of equilibrium in the particular environment.

The addition of Grignard reagents to α,β-unsaturated systems affords a comparison of the relative rates of 1,2- and 1,4-addition, as the reactions are not reversible and both adducts are stable. The reactions have been studied extensively by Kohler and his collaborators and a few of their data are shown in Table XXII. The results indicate that 1,4-addition increases as the reactivity of the carbonyl system toward R—MgX diminishes. Thus, in the series shown below the reactivity toward Grignard reagents diminishes from R—CO—H to R—CO—N(C₂H₅)₂, but the tendency of the α,β-unsaturated carbonyl

TABLE XXII

ADDITION OF C₆H₅MgBr TO α,β-UNSATURATED CARBONYL SYSTEMS

	Per Cent 1,4-Adduct		Per Cent 1,4-Adduct
CH_2=CH—CO—H..............	0	CH_2=CH—CO—C_6H_5..........	100
CH_3—CH=CH—CO—CH_3.......	40	CH_3—CH=CH—CO—C_6H_5.. ...	100
C_6H_5—CH=CH—CO—CH_3......	12	C_6H_5—CH=CH—CO—C_6H_5....	94
C_6H_5CH=CH—CO—C_2H_5.....	40	$(C_6H_5)_2$C=CH—CO—C_6H_5......	0
C_6H_5CH=CH—CO—OC_2H_5.... .	50	$(CH_3)_2$C=CH—CO—CH_3.......	0
C_6H_5CH=CH—CO—NR_2... ..	90	C_6H_5—C≡C—CO—C_6H_5.......	0

systems to undergo 1,4-addition diminishes in the opposite direction, from R—CH=CH—CO—N(C_2H_5)₂ to R—CH=CH—CO—H.

—CO—H —CO—CH₃ —CO—C_6H_5 —CO—Cl —CO—OC_2H_5 —CO—N(C_2H_5)₂

The effect of a hydrocarbon group attached in the β-position is relatively slight, but the presence of two hydrocarbon groups on the β-carbon impedes 1,4-addition. It is of interest to note that an acetylenic system such as C_6H_5—C≡C—CO—C_6H_5 does not undergo conjugate addition of Grignard reagents.

Robinson has pointed out that the enhanced acceptor activity of the terminal carbon atom in "katio-enoid" structures facilitates the exchange of anions in these systems; the increased activity of aryl halides containing a nitro or carbonyl group in the *ortho-* or *para-*positions and the hydrolysis of *p*-nitrosodimethylaniline are explained by the following mechanisms:
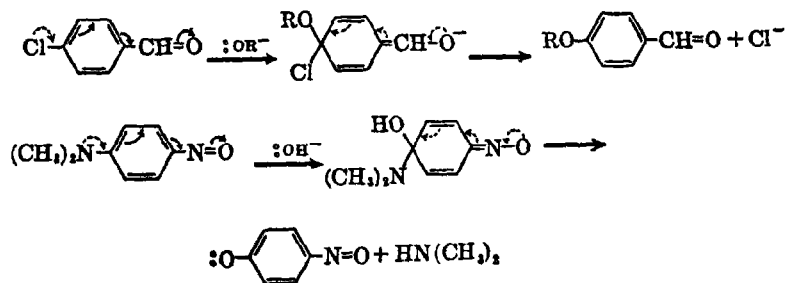
It is evident that the same groups in the *meta*-position will not function in a similar manner owing to the inability of the system to transfer an electronic deficit to the *meta*-position.

The presence of hydroxyl or amino substituents in the β-position of an α,β-unsaturated carbonyl group gives rise to an internal compensation resulting from the effect of dynamic electron-release within the

hetero-enoid system. The dynamic isomerism of unsymmetrical enols, reduced carbonyl activity of p-methoxybenzaldehyde and p-dimethyl-aminoaryl ketones, and the relative inactivity of the corresponding nitriles toward RMgX are typical examples.

$$\underset{A \quad\ B}{H-\overset{+}{O}\overset{\frown}{=}\overset{\frown}{C}=CH\overset{}{\cdot}C\overset{\frown}{=}\overset{\cdot}{O}} \qquad \underset{A \quad\ B}{H-\overset{\delta+}{O}=C-CH=\overset{\delta-}{C}-\overset{}{O}} \qquad \underset{A \quad\ B}{O=C-CH=C-OH}$$

$$CH_3-O\!\!\!\!\diagdown\!\!\!\diagup\!\!\!\!\diagup CH=O \;\rightleftarrows\; CH_3-O\!\!\!\!\diagdown\!\!\!\diagup\!\!\!\!\diagup CH-O\!:$$

$$(CH_3)_2N\!\!\!\!\diagdown\!\!\!\diagup\!\!\!\!\diagup C\overset{\frown}{=}N \;\rightleftarrows\; (CH_3)_2N\!\!\!\!\diagdown\!\!\!\diagup\!\!\!\!\diagup C\overset{\frown}{=}N\!:$$
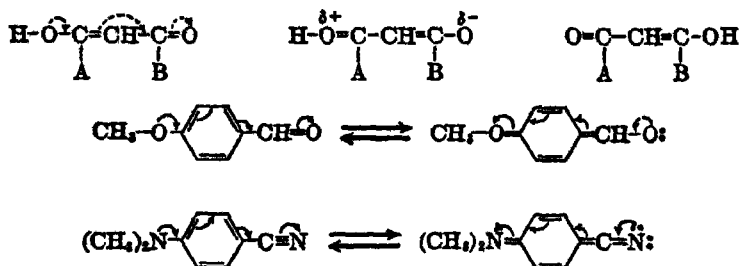
The order of effectiveness of various p-substituents in bringing about internal neutralization is the same as that given under hetero-enoid systems (p. 1909). It must be recognized that these dynamic effects act largely to reduce the rate of carbonyl reactivity and in this way may favor an alternate course of reaction, such as replacement of the β-substituent (cf. preceding paragraph).

Quinonoid Systems. Two carbonyl groups, or similar types, united directly or by means of an intervening ethylenic system, give rise to *ortho*- and *para*-quinonoid structures. Owing to the tendency of the two groups to promote electromeric changes in opposed directions, these systems are highly reactive and the units frequently function independently in their reactions.

1,2-Quinonoid types

$$\underset{R \quad R}{O=C-C=O}$$

1,4-Quinonoid types

$$\underset{R \qquad\qquad R}{O=C-HC=CH-C=O}$$

C_6H_5—CO—CO—C_6H_5
C_6H_5—CO—CO—OR
RO—CO—CO—OR

C_6H_5—CO—CH=CH—CO—C_6H_5
C_6H_5—CO—CH=CH—CO—OR
RO—CO—CH=CH—CO—OR
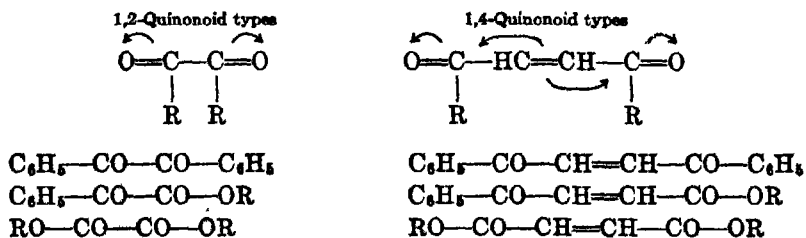
In addition to the true *ortho*- and *para*-benzoquinones, this group includes simple 1,2-dicarbonyl compounds (glyoxal, biacetyl, benzil, ethyl oxalate, and α-ketonic acids) as well as 1,2-dicarbonyl derivatives of ethylene (dibenzoylethylene, benzoylacrylic acid, maleic acid, and citraconic acid).

The strong activating influence of the —CO—CO$_2$R group on an adjacent methylene group, and the citraconic-itaconic acid rearrangement, may be regarded as manifestations of the tendency of a quinonoid

system to revert to one in which the tension of the opposed electromeric effects has been relieved.

$$CH_3-C-CO_2R \qquad \longrightarrow \qquad CH_2=C-CO_2R$$
$$\overset{\|}{O} \qquad\qquad\qquad\qquad \overset{|}{OH}$$

$$CH_3-C-CO_2H \qquad \overset{NaOH}{\longrightarrow} \qquad CH_2=C-CO_2H$$
$$H-\overset{\|}{C}-CO_2H \qquad\qquad\qquad CH_2-CO_2H$$
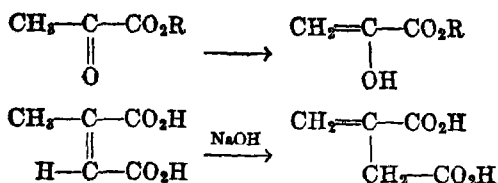
The enhanced reactivity of quinonoid systems is illustrated by the facile addition of acids to *p*-benzoquinone, addition of 1,3-dienes to quinones and to maleic anhydride (Diels-Alder reaction), and the conversion of aryl 1,2-diketones into benzilic acids by means of alkalies.

The addition of acids is probably initiated by fixation of a proton at the carbonyl group, resulting in the development of an active acceptor center in the β-position. Combination of a donor reagent at this point is followed by isomerization to a substituted hydroquinone. However, the Diels-Alder reaction, benzilic acid rearrangement, and condensation reactions occurring in alkaline media may be regarded as a direct attack by an active donor center of the reagent.
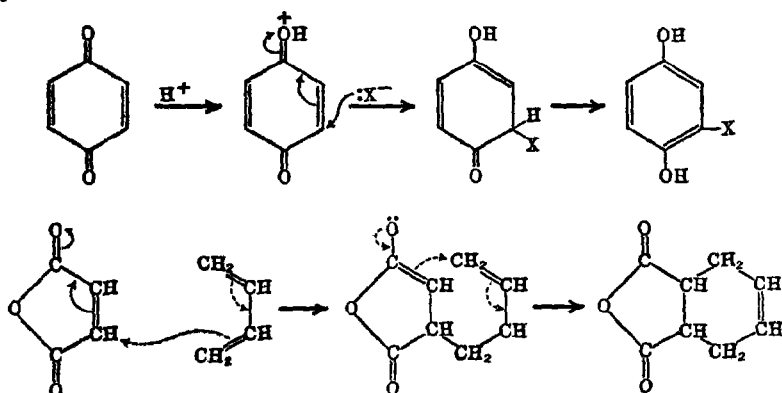
Robinson has given an interesting example of the effect of neutralized systems on the 1,2-diketone group. Glyoxal is a colored substance

Colored and reactive

Colorless and less reactive
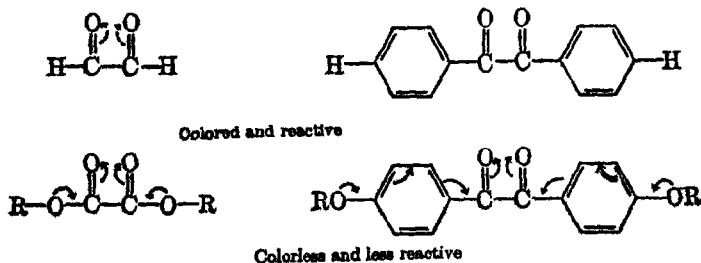
(yellow solid, green vapor) and is highly reactive as a carbonyl compound, whereas ethyl oxalate is colorless and far less reactive. The same relationship holds true for benzil and p,p'-diethoxybenzil; the former is colored and reactive, the latter is colorless and much less reactive. This analogy affords a striking illustration of the principle of vinylogy (pp. 633, 1909).

Peroxidic Systems. The direct union of amino and hydroxyl groups with each other, or with halogen atoms, gives rise to discordant systems of type opposite to the quinones. The former develop an active donor center and the latter an acceptor center. In the simple peroxidic systems such as hydrogen peroxide, hydroxylamine, and hypochlorous acid, α,β-proton migration may give rise to a tautomeric relationship (see dyad systems, p. 1936).

$$H_2N\text{—}NH_2 \qquad H_2N\text{—}OH \qquad HO\text{—}OH \qquad Cl\text{—}OH$$
$$\updownarrow \qquad\qquad \updownarrow \qquad\qquad \updownarrow \qquad\qquad \updownarrow$$
$$\overset{+}{H_3N}\text{—}\overset{..}{\underset{..}{N}}H \qquad \overset{+}{H_3N}\text{—}\overset{..}{\underset{..}{O}}: \qquad \overset{+}{H_2O}\text{—}\overset{..}{\underset{..}{O}}: \qquad H\text{—}\overset{+}{Cl}\text{—}\overset{..}{\underset{..}{O}}:$$
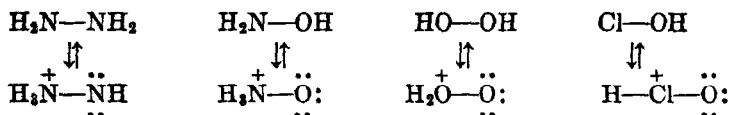
A similar dynamic isomerism is possible in the partially substituted derivatives such as mono-, di-, and trisubstituted hydrazines, N-substituted hydroxylamines, and peracids. This group of compounds is characterized by an ability to act either as oxidizing or reducing agents, according to the nature of the environment; many of them undergo disproportionation reactions involving mutual oxidation and reduction.

$$2C_6H_5\text{—}NH\text{—}NH\text{—}C_6H_5 \rightarrow C_6H_5\text{—}N\text{=}N\text{—}C_6H_5 + 2C_6H_5NH_2$$

$$2C_6H_5\text{—}CO_2\text{—}OH \rightarrow O\text{=}O + 2C_6H_5CO_2H$$
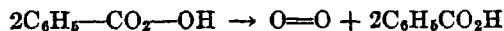
The disproportionation of hydrogen peroxide into water and molecular oxygen is paralleled in the organic derivatives by the corresponding reaction of perbenzoic acid and by the conversion of hydrazobenzene into azobenzene and aniline.[95] The facile conversion of β-phenylhydroxylamine into p-aminophenol and of hydrazobenzene into benzidine, under the influence of strong acids, affords a further illustration of the instability of these systems. The fact that rearrangement of peroxidic systems is brought about by acids, and quinonoid systems by alkalies, is a direct consequence of their respective donor and acceptor activity.

Typical additive processes such as the formation of oximes and hydrazones, and oxidation by peracids, are probably initiated by attack

[95] Wieland, *Ber.*, **48**, 1098 (1915); see, also, Kenner and Knight, *Ber.*, **69**, 341 (1936).

of an external acceptor center by an unshared electron pair of the peroxidic system.

$$R—CH{=}O \begin{cases} \overset{\bar{O}}{\underset{|}{R}}—CH—\overset{+}{N}H_2—OH \to R—\overset{OH}{\underset{|}{C}}H—NH—OH \\ \qquad\qquad\qquad\qquad\qquad \to R—CH{=}N—OH + H_2O \\ \qquad\qquad\qquad \text{Oxime formation} \\ \\ \overset{\bar{O}}{\underset{|}{R}}—CH—\overset{+}{O}H—OCO—R \to R—\overset{OH}{\underset{|}{C}}H—O—OCO—R \\ \qquad \text{Oxidation by peracid} \\ \qquad\qquad\qquad\qquad\qquad\qquad \to 2R—CO_2H \end{cases}$$

$$R_3C—H + HO—Cl \to R_3C—H{\leftarrow}O{\overset{H}{\underset{Cl}{\big\langle}}}{}^{\delta+} \to H_2O + Cl—CR_3$$

$$\text{Chlorination}$$

The view that addition of hypohalous acids and alkyl hypochlorites to olefins proceeds by a similar mechanism (a) is not generally accepted. Recent kinetic studies of addition reactions of stilbene [96] indicate a stepwise process (b) in which an active intermediate is produced by combination of a donor center of the olefin with an acceptor of the addendum ("positive bromine"). The composition of the adduct is determined by competitive reactions of the labile intermediate with an active donor center of the environment ($CH_3—OH$ or Br^-).

It is difficult to reconcile this formulation (b) with the observation that stilbene and isostilbene yield different stereoisomeric adducts, since true carbonium cations are considered to be configuratively unstable [97] and would lead to identical stereoisomers from the cis- and trans-stilbene. The formulation of a carbanion intermediate appears to afford a more satisfactory explanation of the relevant experimental evidence.[98] On

[96] Bartlett and Tarbell, J. Am. Chem. Soc., 58, 466 (1936); 59, 407 (1937).
[97] Wallis and Adams, ibid., 55, 3838 (1933).
[98] Ogg, ibid., 57, 2727 (1935).

the other hand, it is difficult to account for the formation of chloro- and bromo-β-lactones by the addition of chlorine and bromine to aqueous solutions of salts of dimethylmaleic and dimethylfumaric acid [98] without recourse to the hypothesis that the positive fragment of the halogen molecule is added as the first step. Further work in this field will be of considerable interest. There is, of course, no reason to expect that the intimate mechanism of olefinic addition must be the same for all olefins, or for all addenda.
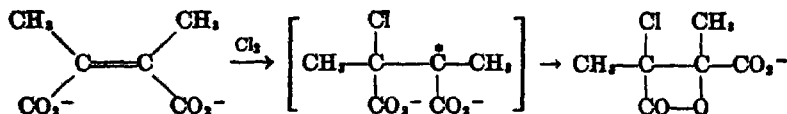
The mechanism of oxidation and reduction by peroxidic structures affords an interesting and difficult problem. Owing to the tautomeric character of the typical systems and their interaction with acids, bases, and hydroxylic solvents, a number of reactive species may be involved.
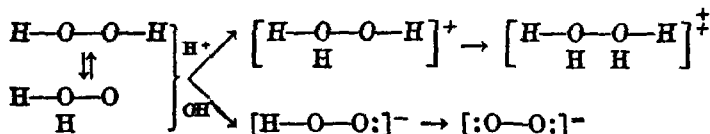
The importance of hydroxylic solvents is indicated by Wieland's observation that peracetic acid does not attack dry acetaldehyde but does attack it in the presence of water; however, this difference is not observed with benzaldehyde. Wieland [99] considers that hydrogen peroxide acts as a hydrogenating (reducing) agent owing to its ability to decompose into molecular oxygen and monatomic hydrogen, and acts as a dehydrogenating (oxidizing) agent through combination with monatomic hydrogen to form water.

An extension of Wieland's view by Bancroft and Murphy [100] involves the postulation of a reversible dissociation and the production of an activated form of oxygen. When hydrogen peroxide acts as an oxidizing agent, the active oxygen is assumed to react both with the substance

$$H—O—O—H \rightleftharpoons 2H\cdot + \cdot O—O\cdot$$

$$H—O—O—H + 2H\cdot \rightarrow 2H_2O$$

$$2H\cdot + \cdot O=O\cdot + HI \rightarrow H_2O + HO—I$$

to be oxidized and with the monatomic hydrogen. They have found that the true electromotive force of hydrogen peroxide in approximately molar acid solutions is about $E_h = + 1.16 \pm 0.3$ volt, and in molar

[99] Wieland, *Ber.*, **54**, 2361 (1921).
[100] Bancroft and Murphy, *J. Phys. Chem.*, **39**, 377 (1935).

potassium hydroxide solutions about $E_h = +0.30 \pm 0.03$ volt. The data indicate that oxidizing and reducing agents having a larger E_h value than hydrogen peroxide (under the given conditions) are reduced by it, and those having a smaller E_h value are oxidized.

Raikow [101] believes that a substance cannot have the same formula as a reducing agent and an oxidizing agent; he attributes reducing action to the normal form HO—OH, and oxidizing action to the oxonium structure H_2O—O. It is pointed out that reductions by hydrogen peroxide are rapid reactions whereas oxidations are slow; these facts are explained on the assumption that the oxonium tautomer is present in small concentrations and is formed from the normal structure by a slow reaction. This hypothesis does not take into account the circumstance that either tautomer gives rise to the same cation or anion. Nevertheless there is evidence that tautomeric phenomena are involved in the reactions of peroxidic systems, since disubstituted organic peroxides such as dibenzoyl peroxide and diethyl peroxide are much less active than perbenzoic acid and ethyl hydroperoxide.

The tautomeric forms of hydrogen peroxide and the monovalent ions possess a donor and an acceptor center, and can enter into reaction by coördination processes. The mode of reaction and observed catalytic effects may be associated with a specific orientation of the coördination mechanism, but the prevalent view is that oxidation-reduction reactions in aqueous solutions involve free radicals and proceed by means of a chain. The mechanism for aldehyde oxidation proposed by Haber and Willstätter,[102] and subsequently modified by others, envisages the following steps: (a) formation of an active free radical, by the intervention of an atom or molecule containing an unshared electron, or by separation of an electron pair; (b) reaction of the free radical with oxygen to form a peroxidic radical; (c) interaction of the latter with the substrate to form the oxidation product and regenerate the original free radical.* The chain is broken when two similar radicals react, or when two unlike radicals react to form the addition product. An inhibitor

(a) CH_3—CH=O + Fe^{+++} → CH_3—$\dot{C}$=O + H^+ + Fe^{++}

(b) CH_3—$\dot{C}$=O + O_2 → CH_3—CO—O—O·

(c) CH_3—CO—O—O· + CH_3—CH=O → CH_3—CO—O—OH + CH_3—$\dot{C}$=O

(d) CH_3—CO—O—O· + HO—C_6H_4—OH → CH_3—CO—O—OH + HO—C_6H_4—O·

(e) HO—C_6H_4—O· + H^+ + Fe^{++} → HO—C_6H_4—OH + Fe^{+++}
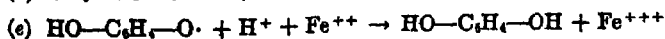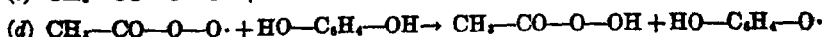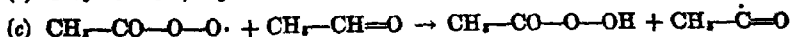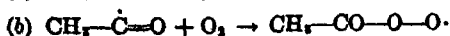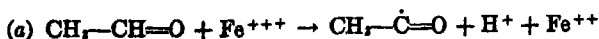
[101] Raikow, Z. anorg. allgem. Chem., 168, 297 (1928); 189, 36 (1930).

[102] Haber and Willstätter, Ber., 64, 2844 (1931).

* For detailed mechanisms for chain reactions of this type and modifications of the Haber-Willstätter mechanism, see Wieland and Richter, Ann., 486, 226 (1931); 495, 284 (1932); and Rice and Rice, "The Aliphatic Free Radicals," Johns Hopkins Press, Baltimore (1935), pp. 170–181.

such as hydroquinone may interrupt the chain through diversion of the peroxidic free radical in a reaction (*d*) which does not regenerate the free radical of the aldehyde, or by direct diversion of the original free radical. The inhibitor may be regenerated by interaction with the accessory products of the initial activation (*e*).

It is significant that aromatic systems, containing hydroxyl and amino substituents in an *ortho* or *para* relationship, are among the most powerful auto-oxidation inhibitors.[103] These compounds may be regarded as vinylogs of the parent inorganic structures, HO—OH, NH$_2$—OH and NH$_2$—NH$_2$. In the organic types the discordant systems can be relieved by tautomerism of a sort that is different from that of the parent structures.

"Imino-alcohol" forms   "Enolamine" forms   "Ketoamine" forms
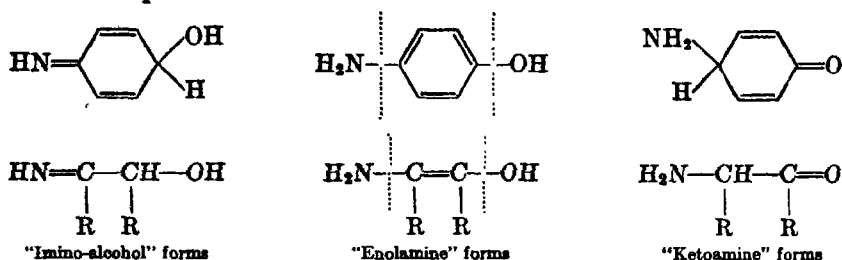
The "enediol" and "enolamine" forms of α-hydroxy and α-amino carbonyl structures may be regarded as vinylogs of the peroxidic systems HO—OH and NH$_2$—OH, and this analogy makes possible an interesting correlation of their behavior in biological reactions. Thus, the relation between an α-amino acid and the corresponding α-keto acid is analogous to that of an aminophenol and the corresponding quinone; the α-hydroxy and α-keto acids become analogous to hydroquinone and quinone, which in turn correspond to HO—OH and O=O.

FREE RADICALS

A free radical is a molecular species, usually electrically neutral, in which is present an atom bearing a single unshared electron. Such structures contain an uneven number of valence electrons and have been designated by Lewis[1] as "odd molecules." Free radicals are therefore an exception to the most fundamental principle of chemical combination ("rule of two"), and they exemplify the highest degree of molecular unsaturation (p. 582).

Free radicals resemble free atoms, such as monatomic hydrogen and chlorine, or alkali metals. Although the notion of organic radicals goes

[103] Moureu and Dufraisse, *Chem. Rev.*, **3**, 113 (1927); Milas, *ibid.*, **10**, 296 (1932).

back as far as Lavoisier, the first experimental evidence of their real existence was given by Gomberg's discovery [104] of triphenylmethyl in 1900. The parent substance hexaphenylethane is capable of reversible dissociation either into neutral free radicals or into ions, and in this respect bears a strong formal resemblance to molecular iodine.

$$(C_6H_5)_3C \text{------} C(C_6H_5)_3 \qquad\qquad I\text{---}I$$

$$2(C_6H_5)_3C \cdot \qquad [(C_6H_5)_3C]^+ [:C(C_6H_5)]^- \qquad 2I\cdot \qquad [:\ddot{I}]^+ [:\ddot{I}:]^-$$

Radicals	Ions		Atoms	Ions
I	II	III		
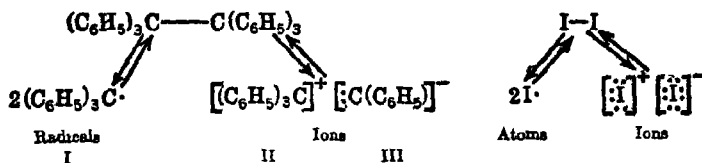
Subsequently, a large number of free radicals of the triarylmethyl type have been prepared and also free radicals containing unsaturated atoms of nitrogen, arsenic, oxygen, sulfur, tin, and lead (pp. 618–619).

A satisfactory interpretation of the mass of experimental observations on free radicals of the triarylmethyl type requires a recognition of the existence of three definite species: electrically neutral triarylmethyl radicals (I) and triarylmethyl cations (II) and anions (III). The free radical is formed alone in non-ionizing solvents, and the ions in ionizing solvents. Triarylmethyl cations are present alone (probably in a solvated state) in solutions of triarylmethyl halides in liquid sulfur dioxide; the corresponding anions exist alone in solutions of alkali metal salts in liquid ammonia.

$$R_3C\text{---}Cl \xrightarrow{\text{(in SO}_2\text{)}} [R_3C]^+Cl^- \qquad\qquad K\cdot + \cdot CR_3 \xrightarrow{\text{(in NH}_3\text{)}} K^+[:CR_3]^-$$

The triphenylmethyl anion is dark red and is the most highly colored of the three species; the free radical is yellow, and the cation is either colorless or yellow. Experiments of Wallis and Adams [97] indicate that an unsymmetrically substituted triarylmethyl anion can exist in an optically active state, but that the corresponding cation undergoes racemization rapidly (p. 398).

The ability of aryl groups to increase the capacity of an attached atom to absorb an electronic deficit or excess (p. 1895) accounts for the effect of aryl groups in stabilizing the ions of opposite sign formed by ionic dissociation. The power of aryl groups to stabilize the neutral radical containing a single unshared electron is due to the same fundamental property—their ability to distribute the singlet to a large number of positions in the system (resonance). This view accounts for the fact that polynuclear aromatic systems are more effective than phenyl

[104] Gomberg, *J. Am. Chem. Soc.*, **22**, 757 (1900); **36**, 1144 (1914); *Chem. Rev.*, **1**, 91 (1924); **2**, 301 (1925).

in stabilizing the radical, and the theoretical sequence—α-naphthyl > β-napthyl > p-xenyl > phenyl—is in agreement with the known facts. The presence of substituents having a considerable +I effect and a −T effect (alkoxyl, halogens) should also increase the extent of dissociation, and this is found to be true.

Ingold [105] holds the view that "the triphenylmethyl anion is obviously much less stable than the kation." As an illustration of the stability of the cation is cited the observation [106] that triphenylmethyl chloride is soluble in liquid ammonia with only slight, and reversible, conversion into the corresponding amine (ammonolysis):

$$[R_3C]^+Cl^- + 2NH_3 \rightleftharpoons R_3C—NH_2 + [NH_4]^+Cl^-$$

Ingold's view appears to ignore the circumstance that triarylmethyl cations are actually formed only in donor solvents (NH_3, SO_2) capable of stabilizing the cation by solvation, whereas the triphenylmethyl anion exists as such in solvents and in the solid alkali metal salts.

$$[R_3C]^+ + SO_2 \rightleftharpoons [R_3C—SO_2]^+ \quad or \quad [R_3C—O—S=O]^+$$
$$[R_3C]^+ + :NH_3 \rightleftharpoons [R_3C—NH_3]^+$$

Evidence that hydrocarbon anions are actually more stable than the corresponding cation is afforded by the fact that in many cases a cation undergoes internal stabilization by intramolecular rearrangement whereas the corresponding anion does not. The relatively greater configurational stability of the triaryl anions, already cited, may be considered to throw further doubt upon the view expressed by Ingold.

Even relatively stable free radicals, such as the triarylmethyls, are extremely reactive substances. They react readily with alkali metals, molecular oxygen, iodine, nitric oxide, and other free radicals; with ethers, esters, ketones, nitriles, and hydrocarbons they form additive compounds involving one molecule of the substrate and two of the free radical. Studies of the velocity of dissociation of hexaphenylethane by the addition of a reagent (halogens, nitric oxide) that reacts instantly with the free radical show that the reaction is strictly unimolecular and is almost independent of the solvent.[99] The period of half-change of hexaphenylethane was found to be 3.3 minutes.

The behavior of hexaphenylethane toward oxygen [107] was found to involve the formation of a labile peroxide which gives rise to chain reactions.

[105] Ingold, *Ann. Repts. Chem. Soc.* (*London*), **25**, 155 (1928)

[106] Kraus and Rosen, *J. Am. Chem. Soc.*, **47**, 2739 (1925).

[107] Ziegler and Ewald, *Ann.*, **504**, 162 (1933).

$$R_3C—CR_3 \rightleftarrows 2R_3C\cdot$$

$$R_3C\cdot + \cdot O—O\cdot \rightarrow R_3C—O—O\cdot$$

$$R_3C—O—O\cdot + R_3C—CR_3 \rightarrow R_3C—O—O—CR_3 + R_3C\cdot$$
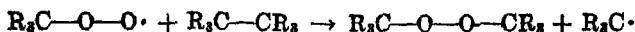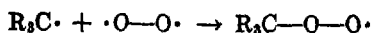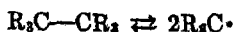
In the presence of an excess of pyrogallol the reaction follows a strictly unimolecular course, and exactly two molecules of oxygen are consumed per molecule of the ethane. The inhibitor functions by its ability to effect an instantaneous fixation of the labile peroxide, converting it to $R_3C—O—OH$.

Free alkyl radicals were first prepared and studied by Paneth [108] in 1929. Free methyl and ethyl radicals were obtained by thermal decomposition of the lead alkyls in a stream of pure hydrogen at low pressures (1–2 mm.). The free alkyls were found to effect direct alkylation of such inactive elements as lead, antimony, zinc, cadmium, bismuth, and tellurium. Further work by Rice and his collaborators [109] has shown that free alkyl radicals react readily with alkali metals, calcium, mercury, lanthanum, thallium, arsenic, and selenium; no alkylation was observed with magnesium, copper, silver, gold, and cerium. The products formed by reaction with arsenic, antimony, and bismuth [110] consist of trialkyls, dialkyls of the cacodyl type, and polymeric monoalkyls (except with bismuth). With tellurium only dimethylditelluride, $CH_3—Te—Te—CH_3$, is formed and no dimethyltelluride, $(CH_3)_2Te$.

The half-life period of the methyl and ethyl radicals is only about 0.006 second, which is even shorter than that of atomic hydrogen under similar conditions (*ca.* 0.1 second). Experimental studies of the higher alkyl radicals * indicates that these decompose readily into methyl or ethyl radicals and olefins. It is estimated that about 75 per cent of the free *n*-butyl radicals formed by the primary thermal decomposition of di-*n*-butylmercury break up into ethylene molecules and ethyl radicals. [109]

A consideration of the reactions of free alkyl radicals with organic molecules (in the gaseous state), based upon the assumption of free radicals in thermal and photochemical decomposition, indicates that they attack the carbon-hydrogen link and not the carbon-carbon link. [109] The thermal or photochemical decomposition of acetaldehyde is inter-

[108] Paneth and Hofeditz, *Ber.*, **62**, 1335 (1929); Paneth and Lautsch, *Ber.*, **64**, 2702 (1931).

[109] Rice and Rice, "The Aliphatic Free Radicals," Johns Hopkins Press, Baltimore (1935).

[110] Paneth, *Trans. Faraday Soc.*, **30**, 179 (1934).

* Evidence for the existence of the *n*-propyl radical has been obtained by Pearson and Purcell, *J. Chem. Soc.*, 253 (1936); its half-life period is estimated to be about 0.002 second, which is only one-third that of the methyl or ethyl radical.

preted from a free radical standpoint by the following chain mechanism (I):

I $$CH_3\cdot + CH_3{-}CH{=}O \rightarrow CH_4 + CH_3{-}\overset{\cdot}{C}{=}O$$

$$CH_3{-}\overset{\cdot}{C}{=}O \rightarrow C{\equiv}O + CH_3\cdot$$
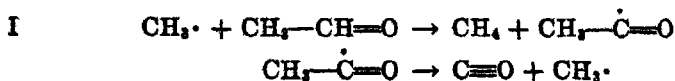
A methyl radical, produced by thermal or photochemical excitation, attacks the C—H link of the aldehyde molecule producing methane and a labile aldehyde radical. The latter decomposes rapidly with loss of carbon monoxide and regenerates a methyl radical, which continues the cycle. It is found experimentally that the products are entirely methane and carbon monoxide. The pyrolysis of acetone to yield ketene and methane (II) is explained by a similar mechanism.[109] But the photo-

II $$CH_3\cdot + CH_3{-}CO{-}CH_3 \rightarrow CH_4 + \cdot CH_2{-}CO{-}CH_3$$

$$\cdot CH_2{-}CO{-}CH_3 \rightarrow CH_2{=}C{=}O + CH_3\cdot$$
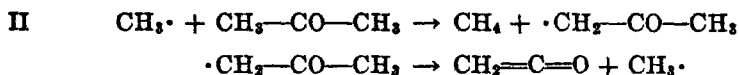
chemical decomposition of acetone yields ethane and carbon monoxide, and it is difficult to account for this difference if both reactions are assumed to occur by way of free methyl radicals.

The possibility of free radicals being formed as intermediate products in the course of chemical reactions in the liquid state or in solutions is only occasionally supported by the experimental evidence. In general, ionic or pseudo ionic mechanisms (p. 1865) are the more common modes of reaction, and free radicals arise only under rather special conditions. There is convincing evidence that thermal and photochemical decompositions occur by way of radical chains. Other reactions in which free radicals may arise are those involving alkali metal atoms (metal ketyls, Wurtz-Fittig reaction), monatomic hydrogen, molecular iodine, molecular oxygen, hydrogen peroxide (and other peroxidic systems), quinones, nitric oxide (and odd molecules in general), atoms and ions of the transition elements, and electrolysis.

The addition of metallic sodium or lithium to arylated olefins (p. 506) and dienes may be cited as an illustration of a reaction involving free radicals. Schlenk and Bergmann [111] found that an atom of sodium initially adds a single electron to the carbon directly attached to the aromatic ring, giving a product analogous to the metal ketyls. The

$$Na\cdot + (C_6H_5)_2C{=}CH_2 \rightarrow Na^+[(C_6H_5)_2\overset{\cdot}{C}{-}\overset{\cdot\cdot}{C}H_2]^- \rightleftarrows [(C_6H_5)_2\overset{\cdot\cdot}{C}{-}\overset{\cdot}{C}H_2]^-$$

$$\rightarrow Na^+[(C_6H_5)_2\overset{\cdot\cdot}{C}{-}CH_2{-}CH_2{-}\overset{\cdot\cdot}{C}(C_6H_5)_2]Na^+$$

$$Na\cdot + (C_6H_5)_2C{=}C(C_6H_5)_2 \rightarrow Na^+[(C_6H_5)_2\overset{\cdot}{C}{-}\overset{\cdot\cdot}{C}(C_6H_5)_2]^-$$

$$\overset{Na\cdot}{\longrightarrow} Na_2^+{}^+[(C_6H_5)_2\overset{\cdot\cdot}{C}{-}\overset{\cdot\cdot}{C}(C_6H_5)_2]^-$$
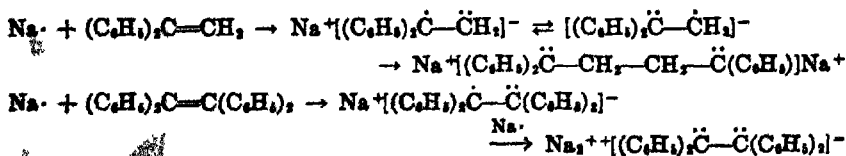
[111] Schlenk and Bergmann, *Ann.*, **463**, 1 (1928).

subsequent course of the reaction is determined by the stability of the initial product. In the case of 1,1-diphenylethylene, two of the units combine to give the disodium derivative of 1,1,4,4-tetraphenylbutane. With tetraphenylethylene, dimerization does not occur but reaction with a second atom of sodium gives the disodium derivative of tetraphenylethane. Conant and Scherp [89] have found that isoprene and 2,3-dimethylbutadiene add two molecules of triphenylmethyl in the 1,4-positions, and this reaction can be formulated in a similar manner; there is also the possibility that it occurs by an ionic mechanism.

$$2(C_6H_5)_3C\cdot + CH_2{=}C{\overset{CH_3}{|}}{-}{\overset{CH_3}{|}}C{=}CH_2$$

$$\rightarrow (C_6H_5)_3C{-}CH_2{-}{\overset{CH_3}{|}}C{=}{\overset{CH_3}{|}}C{-}CH_2{-}C(C_6H_5)_3$$

$$[R_3C\colon]^-Na^+ + CH_2{=}CH{-}CH{=}CH_2$$

$$\rightarrow [R_3C{-}CH_2{-}CH{=}CH{-}CH_2\colon]^-Na^+ + H{-}NEt_2$$

$$\rightarrow R_3C{-}CH_2{-}CH{=}CH{-}CH_3 + Na^+[\colon NEt_2]^-$$
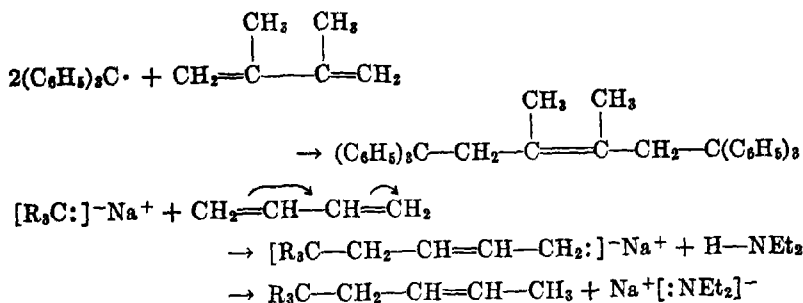
Ziegler and his collaborators [112] have found that alkali metal alkyls are active polymerizing agents for 1,3-dienes. By arresting the polymerization with diethylamine, phenylisopropylpotassium and butadiene gave 1-(phenylisopropyl)-butene. The mechanism of this polymerization is not clear, but if the active reagent is the alkyl ion, the process would follow an ionic mechanism. This view finds some support in the observation that the effectiveness of the alkali alkyls decreases in the order—benzyl > phenylisopropyl > triphenylmethyl—which is the reverse of the anionic stabilities and therefore parallels their donor activity.

The observation that molecular oxygen arrests the photocatalyzed addition of bromine to cinnamic acid [113] suggests that a radical chain occurs in the reaction.

$$Br_2 \rightarrow 2Br\cdot \qquad X{=}Y + Br\cdot \rightarrow X{-}Y{-}Br\cdot$$

$$X{-}Y{-}Br\cdot + Br_2 \rightarrow Br{-}X{-}Y{-}Br + Br\cdot$$

$$\cdot O{-}O\cdot + X{-}Y{-}Br\cdot \rightarrow X{=}Y + \cdot O{-}O{-}Br$$

$$\cdot O{-}O{-}Br + Br\cdot \rightarrow Br_2 + \cdot O{-}O\cdot$$
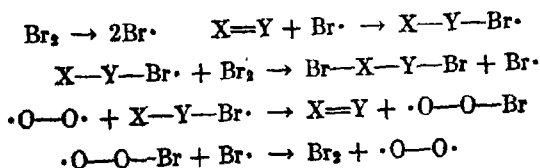
Molecular oxygen may arrest the chain reaction by conversion of the labile bromo-olefin radical (formulated as X—Y—Br·) into the original

[112] Ziegler and collaborators, Ann., 511, 13, 45, 64, 101 (1934).
[113] Daniels and Bauer. J. Am. Chem. Soc., 56, 2014 (1934).

olefin and a stabler bromo-oxygen radical; the latter does not attack the olefin but can react with a bromine atom to regenerate molecular oxygen and bromine. The addition of halogens and halogen acids to olefins in the dark and in the presence of polar catalysts or solvents appears undoubtedly to involve an ionic mechanism (p. 1864), and in these cases molecular oxygen has little or no effect.

In some instances it appears that the olefin itself reacts with molecular oxygen to form a labile peroxide and that the latter may alter the mechanism of addition so as to bring about a reversal of the orientation of addition (p. 638). Studies by Kharasch and his collaborators [72] indicate that the addition of hydrogen bromide to highly purified olefins in the absence of molecular oxygen or peroxides (or in the presence of anti-oxygens) occurs in the direction anticipated from theoretical considerations. This reaction, designated as the normal addition, follows a polar mechanism and usually occurs much less rapidly than the

$$CH_2\!\!=\!\!CHR + HBr \Big\langle \begin{array}{l} CH_3\!\!-\!\!CHBr\!\!-\!\!R \\ \text{Normal reaction (polar)} \\[4pt] CH_2Br\!\!-\!\!CH_2\!\!-\!\!R \\ \text{Peroxide-catalyzed reaction} \end{array}$$
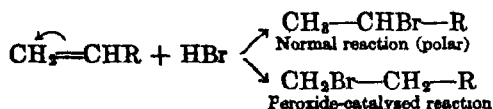
peroxide-catalyzed reaction. It is possible that the peroxide-catalyzed addition occurs through an intermediate radical, but the detailed mechanism is uncertain. The orientation of addition of hydrogen iodide to olefins is not reversed in the presence of peroxides, but this result may be due to the destruction by hydrogen iodide of the original peroxide or of a labile intermediate.

TAUTOMERISM

Tautomeric structures were classified by Laar [114] in 1885 as dyad, triad, tetrad, and pentad systems, depending upon the number of atoms intervening between the initial and final positions of the mobile hydrogen atom. This classification serves as a convenient basis for consideration of their electronic characteristics.[115] The ionic mechanism of tautomeric change (ionotropy) is now clearly established, and the two different types of exchange are distinguished by the terms prototropy and anionotropy. The salient features of prototropic change, without reference to the actual mechanism, are the following: (a) separation of a proton, (b) redistribution of the disengaged electron-pair by electromeric dis-

[114] Laar, *Ber.*, **18**, 648 (1885).

[115] Baker, "Tautomerism," Routledge and Sons, London (1934); see, also, Waters, "Physical Aspects of Organic Chemistry," Routledge and Sons, London (1935); Watson, "Modern Theories of Organic Chemistry," Oxford University Press (1937); Branch and Calvin, "The Theory of Organic Chemistry," Prentice-Hall, New York (1941).

placements in the resulting anion, (c) recombination of a proton at the new anionic center. Anionotropic change has the converse ionic relationship, involving separation of an anion and redistribution of the resulting electronic deficit (open sextet).

As a matter of convenience tautomeric change is formulated frequently as an intramolecular migration, but physical evidence indicates that the process involves an actual separation of the ions concerned. Prototropic change does not take place in the vapor or in the solid state; it is catalyzed by proton-donors and proton-acceptors (acids and bases), and is facilitated by an amphoteric solvent such as water. Brönsted and Guggenheim [116] have formulated the isomeric change in the presence of an amphoteric solvent by the general equation:

$$B: + H—S + HA \rightleftarrows [BH]^+ + S—H + [:A]^-$$
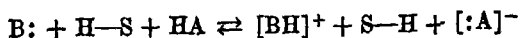
The symbols H—S and S—H are used to indicate the two forms of a prototropic compound; B and HA represent a basic catalyst and its conjugate acid. It will be observed that either isomer should give rise to an identical anion; an essential feature of the tautomeric relationship is a condition of equilibrium between two or more resonating structures (unperturbed states) of the anion.

The condition of resonance may be indicated by writing the structure of one of the final (unperturbed) states and attaching the usual curved arrow symbols showing how the electronic configuration should be modified to represent the actual electronic distribution in the system.[*] The keto-enol systems provide typical illustrations. The conversion of one form of the anion into the other does not require the rearrange-

Keto form   Mesomeric anions   Enol form
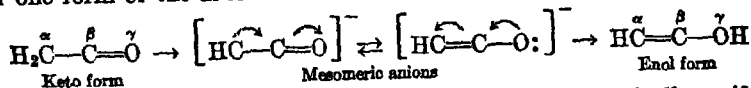
ment of any atomic nuclei, and the anion may react chemically as if it possessed either structure. As the terminal atoms are not identical, the distribution of the anionic charge between α and γ is unequal and will be determined by their relative electron-attraction (effective nuclear charges) and polarizabilities, including the influence of attached groups (internal factors) and of the environment (external factors).

[116] Brönsted and Guggenheim, J. Am. Chem. Soc., 49, 2554 (1927).

[*] Ingold (J. Chem. Soc., 1120 [1930]; Chem. Rev., 15, 225 [1934]) has pointed out that this notation does not distinguish between an electromeric polarizability effect and a mesomeric polarization, for the curved arrows merely denote a mechanism of electronic displacement which is supposed to characterise a molecular state or a process occurring in the course of a reaction. He has introduced curved bond signs without arrows to indicate the "distributed" electron pairs: O—CR—O would represent the mesomeric state of a carboxylate anion rather than O=CR—O.

Dyad Systems. Lapworth [117] pointed out many years ago that tautomerism in odd-numbered systems (triad and pentad) does not alter the valence of any atom, but when an even number of atoms is involved (dyad and tetrad) the valence of one of the terminal atoms is changed by two units. Consequently, dyad and tetrad tautomerism can arise only in systems containing a terminal atom of variable valence, such as nitrogen, oxygen, or sulfur. The typical dyad system, hydrogen cyanide $\rightleftarrows$ isocyanide, was considered to involve bivalent carbon; the

$$\text{H—C} \equiv \text{N} \rightleftarrows \text{C} = \text{N—H} \qquad\qquad \text{H—C} \equiv \text{N}: \rightleftarrows :\overset{-}{\text{C}} \equiv \overset{+}{\text{N}}\text{—H}$$

modern interpretation of this change is based upon the formulation of the isocyanide as an electrical dipole. [77] The electronic theory has led to recognition of the circumstance that dyad and tetrad tautomerism always involves the creation of an electrical dipole and that the valence increase of two units consists of one covalence and one electrovalence. In the cyanide $\rightleftarrows$ isocyanide transformation, 4-covalent carbon becomes 3-covalent and acquires one electrovalence (anionic); 3-covalent nitrogen becomes 4-covalent and acquires one electrovalence (cationic).

Oxime-nitrone tautomerism affords another illustration of valence changes in dyad prototropic systems. This type of tautomerism occurs in relatively few organic compounds but more frequently in inorganic molecules, such as nitrous acid, sulfurous acid, hypochlorous acid, hydroxylamine, hydrazine (see peroxidic systems, p. 1924).

$$\text{R}_2\text{C} = \overset{..}{\text{N}}\text{—OH} \rightleftarrows \text{R}_2\text{C} = \overset{+}{\text{N}}\text{H}\text{—}\overset{-}{\text{O}} \qquad\qquad \text{O} = \overset{..}{\text{N}}\text{—OH} \rightleftarrows \text{O} = \overset{+}{\text{N}}\text{H}\text{—}\overset{-}{\text{O}}$$

<div align="center">Prototropic dyad systems</div>

$$\text{C}_6\text{H}_5\text{—}\overset{\frown}{\overset{..}{\text{N}}} = \overset{\frown}{\text{N}}\text{—OH} \rightleftarrows [\text{C}_6\text{H}_5\text{—}\overset{+}{\text{N}} = \text{N}:] \ [:\text{OH}]^-$$

$$\text{CH}_3\text{—}\overset{..}{\text{N}}\text{—CH}\text{—}\overset{\frown}{\text{OH}} \rightleftarrows \left[\text{CH}_3\text{—}\overset{+}{\text{N}} = \text{CH}\right] [:\text{OH}]^-$$

<div align="center">Anionotropic dyad systems</div>
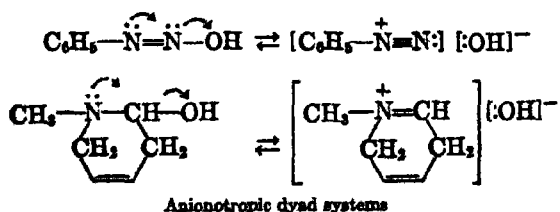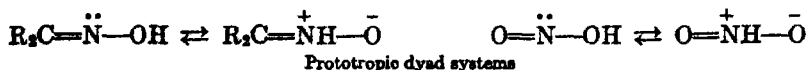
Examples of dyad anionotropic systems are found in the pseudobases encountered frequently in alkaloid chemistry and in the diazonium hydroxides.

Individual isomers of dyads are unknown, and it appears plausible to assume that α,β-migrations can occur spontaneously or, at least, more readily than α,γ-migrations. The apparently spontaneous interchange in dyad systems is probably due to intermolecular association

[117] Lapworth, *J. Chem. Soc.*, **73**, 457 (1898).

involving 2-covalent hydrogen (hydrogen-bridges). Resonance effects in the associated molecules tend to bring about a condition such that

$$R_2C{=}N \underset{H-O}{\overset{O-H}{\diagup\diagdown}} N{=}CR_2 \rightleftarrows R_2C{=}N \underset{H\leftarrow O}{\overset{O\rightarrow H}{\diagup\diagdown}} N{=}CR_2$$

Dimeric "oxime" form   Dimeric "nitrone" form
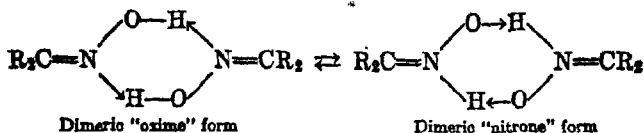
the "tautomeric" forms lose their structural identity. Thus, the distinction between an oxime and a nitrone vanishes when dimerization occurs. Similar resonating forms may be produced also in certain triad prototropic systems, especially those in which the terminal atoms are oxygen and nitrogen (amides, amidines, diazoamino compounds, etc.). It is remarkable that these are the particular cases in which all attempts to separate the individual tautomeric forms have been unsuccessful.

Triad Systems. The best-known examples of tautomerism are the prototropic triad and pentad systems. These may be represented by a general equation, in which the atoms X, Y, and Z may be carbon, nitrogen, or oxygen.

$$\overset{\alpha}{X}{=}\overset{\beta}{Y}{-}\overset{\gamma}{Z}{-}H \rightleftarrows H{-}X{-}Y{=}Z \qquad X{=}Y + Z{-}H \rightarrow H{-}X{-}Y{-}Z$$

Triad prototropy   Addition reaction

A close relationship exists between tautomerism and reversible addition reactions;[118] the mechanism of tautomeric change is an intermolecular process involving proton addition and elimination (p. 1936). The more important types of triad systems are shown in Table XXIII. Pentad systems may be regarded as extensions (vinylogs) of the corresponding triad structures.

TABLE XXIII

TRIAD TAUTOMERIC SYSTEMS

Three carbon	H—C—C=C	C=C—C—H
Keto-enol	H—C—C=O	C=C—O—H
Imino-enamine	H—C—C=N	C=C—N—H
Nitrile-imine	H—C—C≡N	C=C=N—H
Azo-hydrazone	H—C—N=N	C=N—N—H
Nitroso-oxime	H—C—N=O	C=N—O—H
Aci-nitro	H—C—N=O	C=N—O—H
	↓	↓
	O	O
Amide-imidol	H—N—C=O	N=C—O—H
Amidine	H—N—C=N	N=C—N—H
Diazoamino	H—N—N=N	N=N—N—H
Nitrosoamino	H—N—N=O	N=N—O—H
Allylic	X—C—C=C	C=C—C—X

[118] Ingold, *ibid.*, **123**, 1706 (1923).

The energy relationships of tautomers have been examined by Branch and Calvin,[119] who have calculated from bond energies the difference in energy (ΔH) for the tautomeric change of a number of triad systems. These values are only rough approximations since they do not take into account the resonance energies and electrostatic energies. However, the values they have obtained correspond with the generally accepted views that, in simple cases, the ketones and aldehydes are stable with respect to the vinyl alcohols; the amides are stable with respect to the imidols; the aldimines and ketimines are stable with respect to the vinyl amines; the oximes are stable with respect to nitroso compounds; diazohydroxy compounds are stable with respect to nitrosoamines; hydrazones are stable with respect to azo compounds.

TABLE XXIV

ΔH FOR TRIAD TAUTOMERIC CHANGES

(Calculated from bond energies)

$$H-X-Y=Z \rightarrow X=Y-Z-H$$
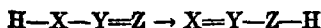
	kcals./mole		kcals./mole
Aldehyde → enol	+15	Nitroso → oxime	−12
Ketone → enol	+18	Diazo → nitrosoamine	− 8
Amide → imidol	+10	Azomethine	0
Imine → enamine	+ 8	Azo → hydrazone	− 9
Three carbon	0	Diazoamino	0
Amidine	0		

Ring-chain tautomerism affords a striking illustration of the analogy between tautomeric change and reversible additive reactions, since the ring form is an obvious addition product. The rings most frequently encountered are five-membered cycles containing one double bond and five- or six-membered saturated cycles; occasionally, three-membered rings occur.
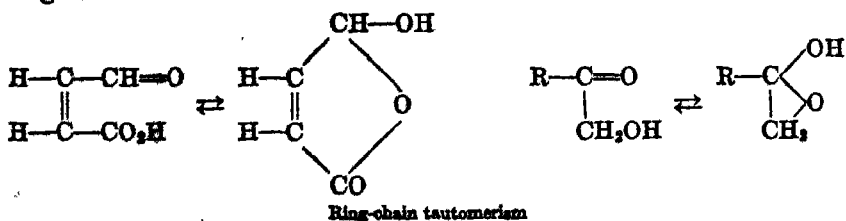
Ring-chain tautomerism

[119] Branch and Calvin, "The Theory of Organic Chemistry," Prentice-Hall, New York (1941).

$$\begin{array}{ccc}
\text{H—C—CO—Cl} & \text{H—C} & \underset{\displaystyle\text{CO}}{\overset{\displaystyle\overset{\displaystyle\overset{\text{Cl}}{\diagup}}{\text{C—Cl}}}{\Big\vert\Big\vert}} \\
\text{H—C—CO—Cl} & \text{H—C}
\end{array}$$

$$R\text{—CH}\!=\!\text{CH—CH}_2\text{—Br}$$
$$\Updownarrow$$
$$R\text{—CHBr—CH}\!=\!\text{CH}_2$$

Anionotropic systems
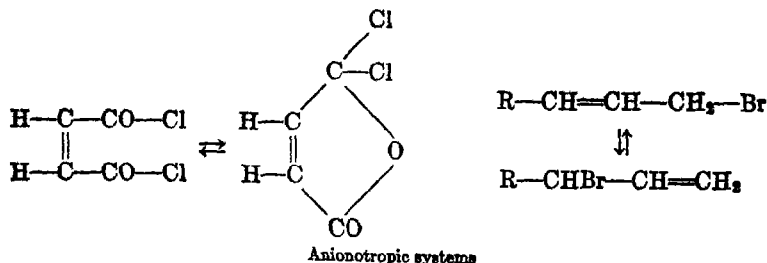
The recognition of anionotropic triad systems is comparatively recent,[81] and the examples are limited almost entirely to the three-carbon (allylic) type. Interconversion of the 1,2- and 1,4-adducts of conjugated dienes, the *sym.*- and *unsym.*-phthalyl chlorides, and derivatives of cinnamyl and phenylallyl alcohols [120] are typical examples. The study of anionotropic change has not yet advanced as far as that of prototropic change, but it is of interest to note that a number of generalizations relating to their mobility and equilibrium, deduced from theoretical considerations, have been verified experimentally. The tendency to migration in the α-phenylallyl—cinnamyl series for different potential anions follows the same sequence as the ionic stability: bromide > acetate > alcohol.

$$\underset{\displaystyle\text{OH}}{\text{C}_6\text{H}_5\text{—CH—CH}\!=\!\text{CH}_2}\qquad\qquad \text{C}_6\text{H}_5\text{—CH}\!=\!\text{CH—CH}_2\text{—OH}$$
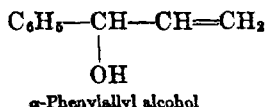
α-Phenylallyl alcohol   Cinnamyl alcohol

The individual alcohols can be obtained separately, and each can be esterified without a change of structure. Conversion of the α-phenylallyl esters to the cinnamyl esters can be effected by heating in a solvent; the rate of conversion varies with the ionizing power (dielectric constant) of the solvent: benzonitrile, acetic anhydride > chlorobenzene > p-xylene. Isomerization of α-phenylallyl bromide is extremely rapid, so that the alcohol yields cinnamyl bromide when treated with hydrobromic and acetic acids. The observed influence of α-substituents upon the mobility of allylic systems is in agreement with the anticipated

$$p\text{-Cl—C}_6\text{H}_4\text{—} \gg \text{CH}_3\text{—C}_6\text{H}_4\text{—} \rightarrow \text{C}_6\text{H}_5\text{—} \gg \text{CH}_3\text{—} \rightarrow \text{H}$$
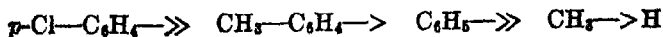
sequence,[100] based upon the view that the mobility is increased by any group which can facilitate electron release ($-I$ or $-T$ effect).

The most effective activating groups for prototropic change will be those that have a strong electron attraction *and* can also provide a

[134] Burton and Ingold, *J. Chem. Soc.*, 904 (1928); Burton, *ibid.*, 248 (1930).

suitable seat for the charge on the electromeric anion. An ammonium group $-NR_3{}^+$, in spite of its powerful electron-attraction $(+I)$, does not satisfy the second requirement and, consequently, has only a weak activating influence. On the other hand, nitrile and carbonyl groups, which satisfy both requirements, have an extremely powerful activating effect. The relative activating effects in a series of carbonyl structures —CO—R will be enhanced by the ability of R to reinforce the electron-attraction of the carbonyl carbon $(+I)$, but will be diminished by an ability of R to furnish electrons by electromeric electron-release (see neutralized systems, p. 1910). On this basis the activating influence of a series of groups, substituted at the α- or γ-position of a triad system, would decrease in the following order:

Activation effects in X=Y—ZH—A=B

A=B is   —CO—CO₂Et> —CO—H> —CO—CH₂> —CO—Cl>

—CO—OCH₂> —CO—NH₂> —CO—O
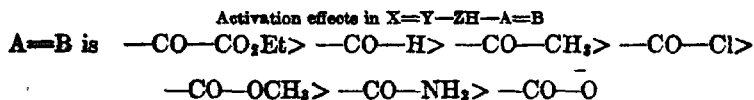
The anticipated order is in excellent agreement with experimental observations of the behavior of prototropic systems. It is of interest to note that substitution at the β-position has much less effect than at the α- or γ-positions. Indeed, if the terminal atoms X and Z in a triad system X=Y—ZH remain constant, variations of Y have but little influence on the mobility of the system.

Pentad Systems. When two activating groups X=Y and A=B are attached to the same atom, there results a pentad system of the general form X=Y—ZH—A=B. Many of the best-known examples of tautomerism fall into this class, which may be regarded as "extended" or "double" triad systems. In the pentad structures the mobility is determined largely by the characteristics of X, Z, and B; the atoms Y and A are much less significant.

In simple keto-enol triads the position of equilibrium is very strongly toward the keto form, and effective enolization is usually brought about through the influence of powerful reagents (strong acids or bases). In general the extent of enolization is greater in the pentad systems, and in many cases the equilibrium mixture contains more than 50 per cent of the enol. The large amount of enol is probably due to the circumstance that the pentad systems permit the formation of chelate structures involving 2-covalent hydrogen (p. 1869) which derive additional stability from resonance effects. The phenols afford an excellent example of a parallel phenomenon; the enol form of a phenol is stabilized through the participation of the C=C in the resonance of the aromatic nucleus.

A survey of the applications of modern electronic theories of chemical reaction reveals that much progress has been made in the direction of correlating the vast subject matter of organic chemistry. The modern theories are more definite in a physical sense and yet are broader in aspect than the former theories. It is evident, of course, that many of the individual postulates and general ideas of the modern theory had existed in the earlier conceptions of Kekulé, Michael, Thiele, Lapworth, Flürscheim, Noyes, Stieglitz, and others. The new theories appear to present the essential truths of the older views in a more precise and unambiguous fashion, to eliminate misconceptions and inconsistencies in the older views, and to bring together many apparently isolated phenomena.

An important contribution of the modern electronic concepts of valence as a basis for the interpretation of reaction mechanisms is this: the imposition, by the introduction of a few fundamental generalizations (especially the principle requiring the maintenance of stable electronic configurations), of certain definite limitations upon the forms of electron displacement which it is permissible to assume in the course of chemical change.

In conclusion it is appropriate to note briefly a few of the significant contributions of the modern theories. The recognition of two kinds of valence forces, electrovalence and covalence, has led to more accurate molecular models of organic systems and has rectified errors in the older structural formulas. The broad concept of electronic resonance (mesomerism) has been of great value in correlating structure and chemical reactivity. The notion that a hydrogen atom can hold two atoms together (2-covalent hydrogen, or hydrogen-bridge) has served to bring together and clarify a large number of experimental observations that had long been regarded as unique or unrelated phenomena.

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CHAPTER 26

THE SIGNIFICANCE OF RESONANCE TO THE NATURE OF THE CHEMICAL BOND AND THE STRUCTURE OF MOLECULES

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CONTENTS

INTRODUCTION

The development of the quantum mechanics during the last decade has led to the clarification of many concepts previously originated by the chemist regarding valence and the nature of the chemical bond, and also to the introduction of some new ideas. Of the latter the most important is the idea of *resonance*, and especially of the *resonance of a molecule among several valence-bond structures*, which, although foreshadowed to some extent by early chemical theories, had not been clearly formulated on the basis of empirical evidence.* In this chapter we shall discuss in a systematic way the essential features of the modern conception of the chemical bond, omitting, however, all quantitative calculations, the quantum-mechanical discussion being restricted to the qualitative description of the results which have been obtained and the discussion of the physical and chemical concepts involved.[1]

The treatment of the chemical bond and the structure of molecules given in this chapter is based largely on the fundamental concept of the shared-electron-pair bond as formulated by G. N. Lewis and developed by many investigators. A description of this development is given in Chapter 25, "Modern Electronic Concepts of Valence" (p. 1821), in which references to the earlier literature are contained.

THE ELECTRONIC STRUCTURE OF ATOMS

During the last twenty-five years a large amount of experimental information has been gathered regarding the structure of atoms, relating to the frequencies and intensities of spectral lines, the magnitudes of resonance and ionization potentials, the behavior of atoms in magnetic and electric fields, etc. This information has, after much effort, been correlated through the development of a theory which seems at present to represent in a completely satisfactory way the extranuclear electronic structure of atoms. This theory, called quantum mechanics or wave mechanics, is a refinement of the old quantum theory. It is not a com-

* The idea of the resonance of molecules among several valence-bond structures, to which a vague resemblance is shown by Kekulé's theory of the benzene ring and Thiele's theory of partial valence [Thiele, *Ann.*, **306**, 87 (1899)], is much more closely approximated by Arndt's theory of intermediate stages [Arndt, Scholz, and Nachtwey, *Ber.*, **57**, 1903 (1924); Arndt, *Ber.*, **63**, 2963 (1930)] and the theory of the mesomeric state developed by the English and American organic chemists [Lowry, *J. Chem. Soc.*, 822, 1866 (1923); Lucas and Jameson, *J. Am. Chem. Soc.*, **46**, 2475 (1924); Robinson and co-workers, *J. Chem. Soc.*, 401 (1926); Ingold and Ingold, *ibid.*, 1310 (1926); see in particular Ingold, *Chem. Rev.*, **15**, 225 (1934)].

[1] See Linus Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, New York (1940), 2nd edition, for a more detailed treatment of the subject.

plete theory of the physical world—it has not been found possible to include within it all the refinements of the theory of relativity, or to extend it to encompass electromagnetic phenomena and the structure of atomic nuclei—but in the field of atomic structure and molecular structure the very extensive agreement between deductions from quantum mechanics and the results of experiment together with the extensive experimental verification of theoretical predictions has caused most theoretical scientists to consider the theory to be generally valid.

In the following paragraphs a brief outline is given of the present views regarding the electronic structure of atoms. The statements made here without support are based upon many experimental facts, but lack of space necessitates their omission.

According to the Bohr theory the electron in the hydrogen atom in its normal state revolves about the nucleus in a circular orbit with radius $a_0 = 0.529$ Å (1 Å $= 1 \times 10^{-8}$ cm.) and the constant speed $v_0 = 2.182 \times 10^8$ cm. per sec. The quantum-mechanical picture is similar but less definite. The state of motion of the electron is represented by an *orbital* (an *orbital wave function*), ψ, obtained by solution of the Schrödinger wave equation. In the physical interpretation of the quantum

FIG. 1.—The probability functions ψ^2 and $4\pi r^2 \psi^2$ for the normal hydrogen atom.

mechanics the square of the wave function, ψ^2, represents the *probability distribution function* for the position of the electron, such that $\psi^2 dV$ is the probability that the electron be found in the volume element dV, and $4\pi r^2 \psi^2 \, dr$ the probability that it be found between the distances r and $r + dr$ from the nucleus. These quantities are shown in Fig. 1, as calculated for the wave function

$$\Psi_{1s} = \frac{1}{\sqrt{\pi a_0^3}} \, e^{-r/a_0}$$

in which r is the distance from the electron to the nucleus. It is seen that the electron is not restricted to the distance a_0 from the nucleus, but that it does remain most of the time at about this distance, which is indeed the value of r at which the radial distribution function has its maximum value. Moreover, the speed of the electron is also not constant, but can be similarly represented by a probability distribution func-

tion, and it is found that the root-mean-square speed has just the Bohr value v_0. The normal hydrogen atom can accordingly be described by saying that the electron moves in and out about the nucleus, with about the speed v_0, in such a way as to remain most of the time within a distance not much greater than a_0. Over a period of time long enough to permit many cycles of motion of the electron the normal hydrogen atom can be described as consisting of the nucleus surrounded by a spherically symmetrical ball of negative electricity (the electron blurred by a time exposure of its rapid motion). The exponential nature of the wave function makes it impossible for us to assign a definite radius to the atom, which fades away gradually with increasing r, but from Fig. 1 it may be said that it has a radius of around $2a_0$ (or $3a_0$), since the chance that the electron gets beyond this distance is small.

The electron itself has a spin (similar to the rotation of the earth about its own axis), and the spin can be oriented in either one of two ways ($+$ or $-$) relative to a specified direction. Only two electrons can occupy the same orbital, and these two only by having their spins opposed (*Pauli exclusion principle*). The normal helium atom consists of two electrons with opposed spins occupying the 1s orbital. In normal atoms containing more electrons the 1s orbital is always occupied in this way by two electrons, which are said in this case to constitute a *completed shell*, the K *shell*. The size (linear dimensions) of the K shell varies about inversely with the effective nuclear charge, the helium atom being about one-half as large as the hydrogen atom, the lithium ion Li^+ about one-third as large, and so on.

The next outer shell is the L *shell*, consisting of the four orbitals $2s$, $2p_x$, $2p_y$, and $2p_z$, of which $2s$ is somewhat more stable than the $2p$'s. In the atoms lithium to neon, electrons are introduced in these orbitals, two electrons in the same orbital having opposed spins; this gives neon a completed L shell of eight electrons. This *electronic configuration* is represented by the symbol $1s^2\, 2s^2\, 2p_x{}^2\, 2p_y{}^2\, 2p_z{}^2$ or $1s^2\, 2s^2\, 2p^6$, the superscript showing the number of electrons occupying the orbital or orbitals. Here the numbers 1 and 2 (for K and L shell, respectively) give the values of the *total quantum number* n, and the letters s and p represent the values of the *azimuthal quantum number* * l (s, p, d, f, etc., corresponding to $l = 0, 1, 2, 3, \cdots, n-1$).

In an atom or monatomic ion the electrons tend not to pair with one

* In the old quantum theory the azimuthal quantum number determined the eccentricity of the elliptical orbit. This interpretation is retained essentially in the quantum mechanics, the s orbital in a given shell being the most eccentric and penetrating most deeply into the core, the p orbitals next, and so on. The greater penetration into the core (the region near the nucleus) leads to greater stability, and thus gives rise to the stability sequence $ns > np > nd$, etc., indicated in Fig. 2.

another (by occupying the same orbital, their spins being opposed), but instead to occupy different orbitals, keeping their spins parallel. For example, in the normal nitrogen atom there are three unpaired electrons. The two most stable orbitals, 1s and 2s, are occupied by pairs, whereas the three next orbitals, $2p_x$, $2p_y$, and $2p_z$, which do not differ in stability,
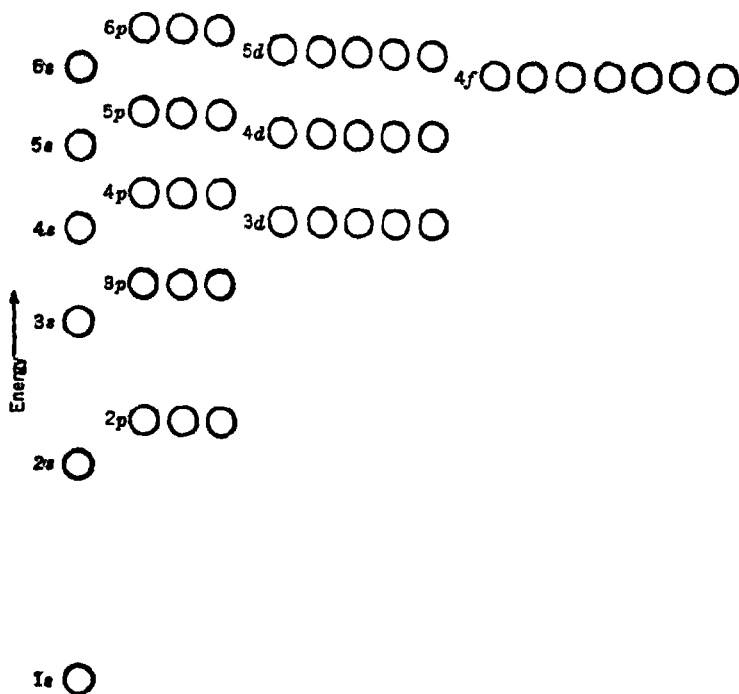
FIG. 2.—The approximate stability sequence for atomic orbitals, the lowest circle representing the most stable orbital (1s). Each circle represents one atomic orbital, which can be occupied either by one electron or by two electrons with opposed spins. In helium the 1s orbital is filled (with two electrons), in neon the 1s, 2s, and three 2p orbitals, and so on.

are occupied by one electron apiece. In oxygen the eighth electron must pair with one of these three in order to enter the L shell, leaving only two unpaired electrons; the same process leads to one unpaired electron in fluorine and none in neon.

There are n^2 orbitals in the shell with total quantum number n, 1 in the K shell, 4 in the L, 9 in the M, 16 in the N, and so on, the numbers of electrons occupying a completed shell being thus $2n^2$. The approximate relative energy values for atomic orbitals are indicated in Fig. 2, the most stable orbitals being the lowest. It is seen that the M shell is not

completely filled with electrons before the N orbitals begin to be occupied. Instead, after the $3s$ and $3p$ orbitals are occupied by an "octet" of eight electrons, giving the stable argon configuration $1s^2 \, 2s^2 \, 2p^6 \, 3s^2 \, 3p^6$, electrons enter the $4s$ orbitals (in potassium and calcium), and only later, in the iron-group transition elements, are the $3d$ orbitals filled by their complement of ten electrons. The palladium and platinum transition elements (ten of each) correspond to filling the five $4d$ and five $5d$ orbitals, respectively, and the rare earths (fourteen) to filling the seven $4f$ orbitals.

It must be mentioned that the stability sequence shown in Fig. 2 is not always strictly applicable. In potassium and calcium the $4s$ orbital
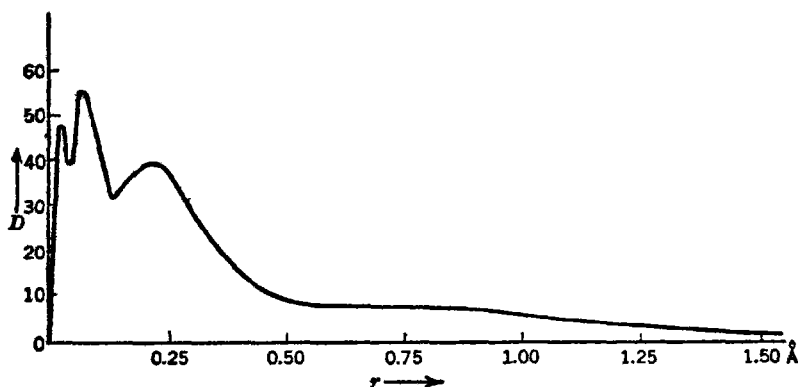
Fig. 3.—The radial electron distribution function for the rubidium ion, showing four electron shells, the outermost being not well defined. (From calculations by Hartree.)

is more stable than the $3d$ orbitals, and hence is occupied by electrons whereas with increase in the atomic number (iron, cobalt, nickel) the $3d$ orbitals become more stable than the $4s$. The same change in relative stability of orbitals takes place in the other transition series.

The outer shell of many stable monatomic ions consists of an octet of eight electrons in s and p orbitals (noble-gas type) or of eighteen electrons in s, p, and d orbitals (eighteen-shell type — Zn^{++}, etc.).

The radial electron distribution function for rubidium ion, with the configuration $1s^2 \, 2s^2 \, 2p^6 \, 3s^2 \, 3p^6 \, 3d^{10} \, 4s^2 \, 4p^6$, is shown in Fig. 3. The K, L, M, and N shells are represented by the successive humps.

ELECTROSTATIC BONDS AND COVALENT BONDS

It is convenient to consider three general classes of chemical bonds: *electrostatic bonds, covalent bonds,* and *metallic bonds.* This classification is not a rigorous one; for although the bonds characteristic of each of the

classes have well-defined properties, especially from the structural view-point, the transition from one class to the other may take place gradually, permitting the existence of bonds of intermediate type (p. 1950). In other cases there may occur a discontinuity in some physical or chemical property, which may be used as a basis for classification (p. 1956). Metallic bonds will not be discussed in this chapter.

The Ionic Bond and Other Electrostatic Bonds. We say that there is a chemical bond between two atoms or groups of atoms if the forces acting between them are such as to lead to the formation of an aggregate with sufficient stability to make it convenient for the chemist to consider it as an independent molecular species. (Thus the weak van der Waals' forces between molecules are not usually considered as leading to the formation of chemical bonds.) If we can assign to each of the atoms or groups of atoms a definite electronic structure, essentially independent of the other atom or group, such that electrostatic interactions lead to strong attraction and the formation of a chemical bond, the bond is said to be an *electrostatic bond*.

The most important of these is the *ionic bond*, resulting from the Coulomb attraction of the excess electric charges of oppositely charged ions. There are essentially ionic bonds between Na^+ and Cl^- in crystalline sodium chloride and in $NaCl$ molecules in sodium chloride vapor.[*] The fluoferriate complex ion, $[FeF_6]^{\equiv}$, consists essentially of Fe^{+++} and F^- ions held together by ionic bonds.

In $[Fe(H_2O)_6]^{+++}$, $[Ni(H_2O)_6]^{++}$, $[Ni(NH_3)_4]^{++}$, and many other complexes the bonds between the central ion and the surrounding molecules are due essentially to the electrostatic attraction of the excess charge of the central ion for the permanent electric dipoles of the molecules.[2] Electrostatic bonds of this type may be called *ion-dipole bonds*. Electrostatic bonds may also result from the attraction of an ion for the induced dipole of a polarizable molecule or from the mutual interaction of the permanent electric dipoles of two molecules.

The Shared-Electron-Pair Bond or Single Covalent Bond. With G. N. Lewis (1916) we write electronic structures such as $H\!:\!H$, $:\!\ddot{C}l\!:\!\ddot{C}l\!:$,

H
$\ddot{}$
$H\!:\!\ddot{C}\!:\!H$, etc., in which only the outer electrons are represented. Here
$\ddot{}$
H

a bond is formed between two atoms by two electrons which are held jointly by the two atoms, and which can be considered as contributing to

[*] For a discussion of ionic bonds in crystals see Pauling, *J. Am. Chem. Soc.*, **51**, 1010 (1929).

[2] Langmuir, *ibid.*, **41**, 868 (1919), especially pp. 930-931.

the outer shell of each. Such a bond is called a *shared-electron-pair bond* or *single covalent bond*.

The nature of these bonds is now well understood as the result of the application of quantum mechanics, beginning with the pioneer work of Heitler and London.[1]

A single covalent bond between two atoms A and B involves two electrons, one orbital from atom A, and one orbital from atom B. One of the electrons has positive spin and one negative spin; the stability of the bond may be considered to result from the interchange of the two electrons between the atoms A and B; that is, from *resonance* between the structures $A \uparrow \ \downarrow B$ and $A \downarrow \ \uparrow B$, the arrows indicating the orientation of the electron spins.

The energy required to separate two atoms joined by a single covalent bond is of the order of magnitude of 50,000 to 100,000 cal. per mole. The strength of the bond depends on the nature of the orbitals involved (p. 1952).

THE IDEA OF RESONANCE [*]

The idea of *resonance*, in its application to chemistry, is the following. If it is possible to write for a molecule (or other system) two or more electronic structures corresponding to about the same energy and satisfying certain other conditions, then no one of the structures alone can be considered to represent the normal state of the molecule, which instead is represented essentially by all of them; and, moreover, the molecule is then more stable (has a smaller energy content) than it would be if it had any one of the structures alone. The molecule is described as *resonating* among various structures, and the energy stabilizing the molecule is called *resonance energy*.

(In quantum-mechanical terms, it is said that the wave function representing the normal state of the molecule is not any one of the wave functions corresponding to the various electronic structures, but is a linear combination of them.)

The principal conditions for resonance are that the structures correspond to the same atomic arrangement (nuclear configuration) and to the same number of unpaired electrons.

The effect of the energy of the structures is the following. If two possible structures have the same energy (and satisfy the other conditions for resonance, mentioned above) the molecule resonates equally

[1] Heitler and London, Z. Physik, 44, 455 (1927).

[*] For a more thorough discussion of this subject see Pauling and Wilson, "Introduction to Quantum Mechanics, with Applications to Chemistry," McGraw-Hill Book Co., New York (1935), Secs. 41, 40f.

between them.' For example, for the nitro group we write the two structures

$$:O\diagdown \underset{+}{N}\diagup \overset{..}{O}^{\overline{.}}: \quad \text{and} \quad :\overset{..}{O}\diagdown \underset{+}{N}\diagup O:$$

The group resonates between these two structures equally, and is thereby made more stable than either one of them. If one of the individual structures is much less stable than the other, its contribution is very small, and resonance makes the molecule only slightly more stable than the more stable of the two structures.

It has already been mentioned that the energy of a single covalent bond between two atoms A and B can be considered as the resonance energy between the two equivalent structures $A \uparrow \quad \downarrow B$ and $A \downarrow \quad \uparrow B$. In the following sections other applications of the idea of resonance will be discussed.

THE COVALENT BOND

The Ionic Character of Covalent Bonds. For a molecule such as HCl we write two reasonable electronic structures, $H:\overset{..}{\underset{..}{Cl}}:$ and $H^+ :\overset{..}{\underset{..}{Cl}}:^{\overline{.}}$, the first corresponding to a *normal covalent bond* between the two atoms (similar to the bonds in H_2 and Cl_2) and the second to an ionic bond. Inasmuch as chlorine is electronegative with respect to hydrogen, we expect the ionic structure, although less stable than the normal covalent structure, to be not far removed from it in energy. These two structures satisfy the conditions for resonance, and the normal HCl molecule must be considered as represented by both of them. The bond is partially covalent and partially ionic, the covalent contribution being the greater. *The bond is stronger than either the normal covalent bond or the ionic bond,* as the result of the resonance energy. It is the stabilizing effect of the partial ionic character which makes covalent bonds between unlike atoms more stable than those between like atoms. A quantitative treatment of the energy of bonds in relation to the relative electronegativity of atoms has been given.[4]

A single bond may lie anywhere between the ionic extreme and the normal covalent extreme. The former extreme is approached closely in CsF, and the latter is reached in bonds between like atoms, as in H_2. In the series of gas molecules HF, HCl, HBr, HI, there is evidence that the ionic character is large in HF (perhaps larger than the covalent character), and that it falls off rapidly in the order HCl, HBr, HI, the last having very little ionic character.

[4] Pauling, *J. Am. Chem. Soc.*, **54**, 3570 (1932); ref. 1, Chapter II.

It must be pointed out that the deduction of bond type from physical properties must be made with great caution. Thus of the fluorides

	NaF	MgF$_2$	AlF$_3$	SiF$_4$	PF$_5$	SF$_6$
M.P.	980°	1400°	1040°	−77°	−83°	−55°C.

those of high melting points have been described as ionic compounds and the others as covalent compounds. Actually the Al–F bond is closely similar to the Si–F bond. The abrupt change in properties between AlF$_3$ and SiF$_4$ is due to a change in atomic arrangement—in the number and distribution of the bonds rather than in their type. In NaF, MgF$_2$, and AlF$_3$ each metal atom or ion is surrounded by six fluorine atoms or ions, to which it is bonded, and each fluorine is bonded to more than one metal (six in NaF, three in MgF$_2$, two in AlF$_3$) in such a way as to make the whole crystal one giant molecule, so that fusion and vaporization can occur only through breaking these strong bonds. In SiF$_4$, PF$_5$, and SF$_6$ crystals there are discrete molecules, each fluorine being bonded only to the central atom; these molecules are held together only by weak van der Waals' forces, and so the substances melt and boil easily. As pointed out long ago by Kossel,[5] this ease of fusion and vaporization would be expected for ionic molecules of high symmetry and is not sound evidence for the presence of covalent bonds. There is strong evidence, such as that mentioned above, that volatility does not depend mainly on bond type, but on the atomic arrangement and the distribution of the bonds.

Bond Orbitals. The Tetrahedral Carbon Atom. An orbital in an atom, such as the s and p orbitals indicated in Fig. 4, can be occupied by one unpaired electron or by two electrons, which form an *unshared pair*. An atomic orbital can also be involved in bond formation, the single covalent bond consisting of the shared pair of electrons occupying two atomic orbitals, one for each atom. These orbitals are conveniently called *bond orbitals*.

A simple quantum-mechanical treatment of the relation between the strengths and relative orientation of the covalent bonds formed by an atom and the nature of its bond orbitals has been given.[6] It has been seen from the foregoing discussion that the stability of a covalent bond is determined by the resonance energy of the two electrons between the two bond orbitals, one for each atom. The examination of the form of the resonance integral shows that the resonance energy increases in magni-

[5] Kossel, *Z. Physik*, **1**, 395 (1920).

[6] Pauling, *J. Am. Chem. Soc.*, **53**, 1367 (1931); ref. 1, Chapter III; see also Slater, *Phys. Rev.*, **37**, 481; **38**, 1109 (1931), and Hultgren, *ibid.*, **40**, 891 (1932).

tude with increase in the *overlapping* of the two bond orbitals (the word
overlapping signifying the extent to which the regions in space in which
the two orbital wave functions have large values coincide). Conse-
quently it is expected that *of two orbitals in an atom the one which can over-
lap more with an orbital of another atom will form the stronger bond, and,*
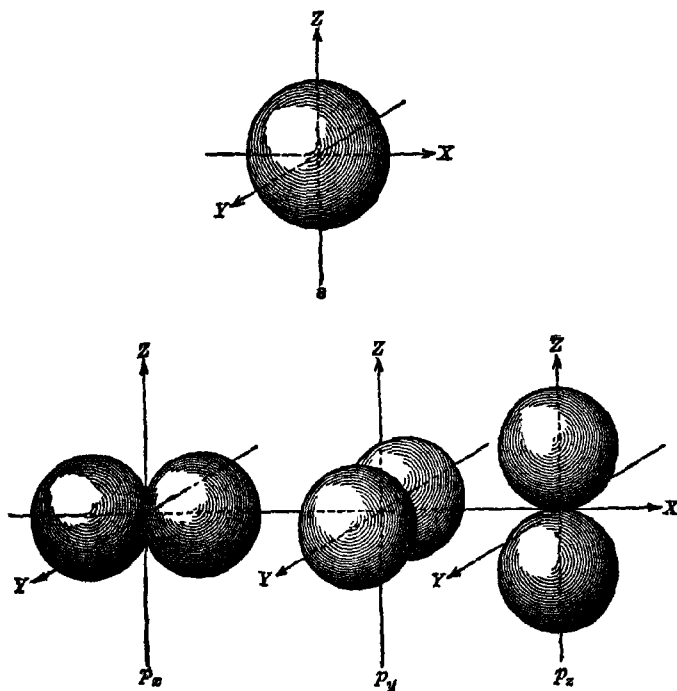
Fig. 4.—Representation of angular dependence of *s* and *p* atomic orbitals. The
magnitude of each wave function, depending on orientation (polar angles ϑ and φ),
is represented for each wave function by a vector drawn from the origin in the direc-
tion ϑ, φ under consideration to the surfaces shown.

*moreover, the bond formed by a given orbital will tend to lie in that direction
in which the orbital is concentrated.*
    The different bond orbitals of a given atom do not differ very much
in their dependence on *r*, but they may show a great difference in their
dependence on ϑ and φ, that is, in their angular distribution. This is
seen from Fig. 4. The *s* orbital is spherically symmetrical, and so can
form a bond in one direction as well as in any other, whereas the three
p orbitals are concentrated along the three Cartesian axes, and will tend

to form bonds in these directions.* Moreover, the p orbitals are concentrated in these directions, having a magnitude $\sqrt{3}$ times as great as the s orbital; hence (because of greater overlapping) *p bonds are stronger than s bonds*. It is convenient to call this magnitude (1.732 for p orbitals, 1 for s orbitals) the *strength of the bond orbital*.
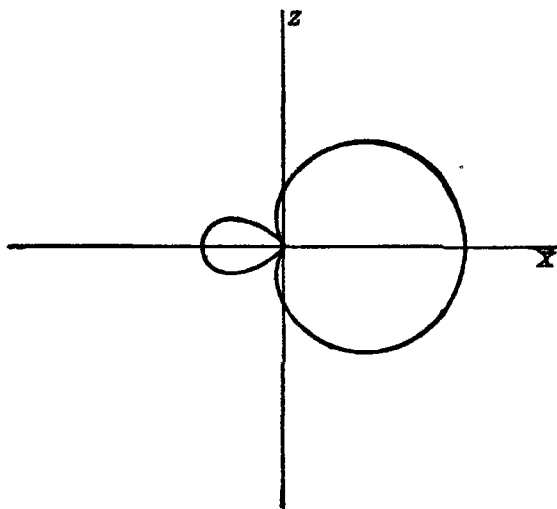
FIG. 5.—Angular dependence of a tetrahedral orbital (with cylindrical symmetry about the x axis).

The conclusion that *p bonds tend to be at right angles* is verified to some extent by experiment. In H_2S, with the electronic structure $:\overset{..}{S}:H$, the
$$\overset{..}{H}$$
bond angle [7] is 92°, and values between 90° and 110° are found in many similar molecules.

This does not mean, however, that the carbon atom will form three p bonds at right angles and a fourth (weaker) bond in some other direction. Instead, by the process of *hybridization* (the formation of linear combinations) of the s and p orbitals four *tetrahedral bond orbitals* can be constructed; these orbitals are the best bond orbitals which can exist in the L shell, having a strength of 2.00 as a result of great concentration in one direction (Fig. 5). The four tetrahedral bond orbitals are mutually equivalent, and they are directed towards the corners of a regular tetra-

---

* The orientation of the axes is of course arbitrary; we should say that the bond directions for the three p orbitals are at right angles to one another.

[7] Cross, *Phys. Rev.*, **47**, 7 (1935).

hedron. The properties which these bond orbitals give to the carbon atom are just those found experimentally, which led the chemist to the concept of the tetrahedral carbon atom (p. 222).

If a first-row element forms four covalent bonds (the maximum possible, as there are only four orbitals in the L shell), these will be tetrahedrally directed, with angles 109° 28′, provided that there is no distortion arising from steric or other effects.* When only two or three bonds are formed the bond angle may lie anywhere between 90° and 109° 28′ (ignoring distortion), inasmuch as two opposing effects are operative. An unshared pair will tend to occupy the stable s orbital, leaving the p orbitals for the formation of bonds at 90° angles. On the other hand, the shared pairs strive to cause hybridization and the formation of tetrahedral bonds with use of the best bond orbitals (strength 2). That these opposing effects reach a compromise is indicated by the intermediate value, 105°, observed for the bond angle in the water molecule. The value 100° ± 3° is also reported for OF_2. In other oxygen compounds somewhat larger values are found, perhaps because of steric repulsion of the large attached atoms or groups: 111° ± 2° in Cl_2O, 111° ± 4° in dimethyl ether, and 110° ± 5° in dioxane. The nitrogen bond angles in NH_3 and other molecules also have values of about 110°.

Other atoms (Ni^{II}, Pd^{II}, Pt^{II}, Cu^{II}, Au^{III}) can form four covalent bonds directed toward the corners of a square, using hybrid bond orbitals formed from one d, one s, and two p orbitals. Atoms such as Fe^{II}, Fe^{III}, Co^{III}, Pd^{IV}, Pt^{IV}, etc., can form six covalent bonds directed toward the corners of an octahedron, using hybrid bond orbitals formed from two d, one s, and three p orbitals.

It is to be emphasized that the quantum-mechanical treatment given above is neither rigorous nor unique. Most of the problems of chemistry are so complicated that they can be attacked in practice only through extreme simplification. The simplifying assumptions can be chosen in any one of a number of ways. Of these ways two in particular are especially reasonable; these correspond to the two general treatments which have been used to the largest extent in the treatment of the electronic structure of molecules, called the *valence-bond method* and the *molecular-orbital method*. Of these two methods the former is the more closely related to the familiar concepts of chemistry, and for this reason our discussion will be restricted to it. Confidence in the results of its application, which might be shaken by realization of its approximate charac-

* Electron-diffraction studies have shown that the Cl-C-Cl angles in methylene chloride and chloroform have the value 111° ± 2°, only slightly different from the tetrahedral angle.

ter, is reinforced strongly by the fact that essentially the same results are obtained by application of the method of molecular orbitals.[*]

The Magnetic Criterion for Bond Type.[5] It has been mentioned (p. 1949) that discontinuities in physical properties sometimes cannot be relied on as indicating a discontinuity in bond type. For certain substances, however, definite evidence regarding the bond type can be obtained by the observation of one property of the molecule, its magnetic moment.

Let us now consider the complex ions $[FeF_6]^{\equiv}$ and $[Fe(CN)_6]^{\equiv}$. If the bonds connecting the iron atom to the six surrounding groups are ionic, these complexes contain the ferric ion, Fe^{+++}, with twenty-three electrons. Of these electrons eighteen occupy the nine most stable orbitals, and the remaining five the five $3d$ orbitals, the electron configuration being $1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 3d^5$. Electrons in atoms or monatomic ions avoid pairing; hence the five $3d$ electrons distribute themselves among the five $3d$ orbitals without pairing, as indicated in Fig. 6. Now each unpaired electron makes a large contribution to the magnetic moment of the complex, because of its spin, so that a complex ion $[FeX_6]^{\equiv}$ containing ionic bonds would have a very large magnetic moment, and a substance containing it would be strongly paramagnetic.

On the other hand, if the iron atom is attached to the six groups by *octahedral covalent bonds two of the 3d orbitals will be involved in bond formation*, together with the $4s$ and the three $4p$ orbitals (Fig. 6), and the five $3d$ electrons will be forced into the three remaining $3d$ orbitals, only one remaining unpaired. This will give rise to a relatively small magnetic moment.

The experimentally determined moment for $[FeF_6]^{\equiv}$ corresponds accurately to five unpaired electrons, and that for $[Fe(CN)_6]^{\equiv}$ to one; hence in the fluoferriate ion the bonds are essentially ionic and in the ferricyanide ion they are essentially covalent.

[*] The following references relate to the development of the two principal methods of treatment of the electronic structure of molecules. Valence-bond method: Heitler and London, *Z. Physik*, **44**, 455 (1927); Heitler, *ibid.*, **46**, 47; **47**, 835 (1928); **51**, 805 (1929); London, *ibid.*, **46**, 455; **50**, 24 (1928); Pauling, *Proc. Natl. Acad. Sci. U. S.*, **14**, 359 (1928); *Chem. Rev.*, **5**, 173 (1928); Slater, *Phys. Rev.*, **37**, 481; **38**, 1109 (1931); Pauling, *J. Am. Chem. Soc.*, **53**, 1367 (1931). Molecular-orbital method: Burrau, *Kgl. Danske Videnskab. Selskab. Math.-fys. Medd.*, **7**, 1 (1927); Lennard-Jones, *Trans. Faraday Soc.*, **25**, 668 (1929); Hund, *Z. Physik*, **51**, 759 (1928); **63**, 719 (1930); **73**, 1, 565 (1931); **74**, 429 (1932); Herzberg, *ibid.*, **57**, 601 (1929); Mulliken, *Chem. Rev.*, **9**, 347 (1931); *Phys. Rev.*, **40**, 55; **41**, 49, 751 (1932); **43**, 279 (1933); *Rev. Modern Phys.*, **4**, 1 (1932); *J. Chem. Phys.*, **1**, 492 (1933); **3**, 375, 506, 514, 517, 564, 573, 586, 635 (1935). The problem of directed valence is discussed in the following papers, in addition to those already mentioned: Van Vleck, *J. Chem. Phys.*, **1**, 177, 219 (1933); **2**, 20, 297 (1934); Penney, *Proc. Roy. Soc. (London)*, **A144**, 166 (1934); *Proc. Phys. Soc. (London)*, **46**, 333 (1934); Penney and Sutherland, *J. Chem. Phys.*, **2**, 492 (1934); *Trans. Faraday Soc.*, **30**, 898 (1934).

Pauling, *J. Am. Chem. Soc.*, **53**, 1367 (1931); ref. 1, Chapter III.

Octahedrally coördinated ferrous complexes are diamagnetic (with no unpaired electrons) if the bonds are essentially covalent, as in the ferrocyanide ion, $[Fe(CN)_6]^{---}$, and strongly paramagnetic (four unpaired electrons) if the bonds are essentially ionic, as in the hydrated ferrous ion, $[Fe(H_2O)_6]^{++}$.

An interesting example of the application of the magnetic method is provided by heme compounds. Ferroheme, the ferrous salt of protoporphyrin, combines with pyridine, nicotine, cyanide ion, denatured globin,
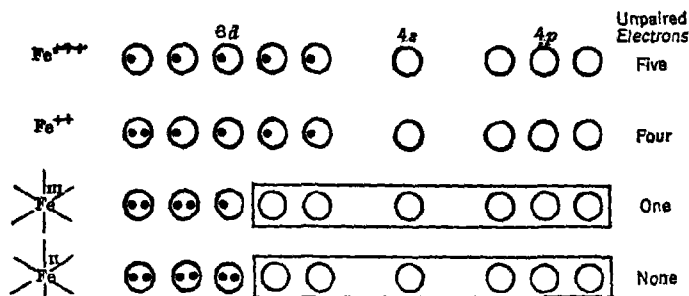
Fig. 6.—Occupancy of orbitals in iron complexes, each large circle representing an orbital and each small circle an electron. The circles enclosed in the rectangles are involved in covalent bond formation.

and other substances to form deep red substances called hemochromogens; the stoichiometric ratio is two groups (pyridine, cyanide ion, etc.) per ferroheme molecule. The structure of the hemochromogens has been determined from magnetic measurements.[9] The substances are diamagnetic, and hence have about the iron atom six covalent bonds which are directed to octahedrally arranged atoms; these are safely presumed to be the four nitrogen atoms of the porphyrin system and two atoms of the attached groups, such as the nitrogen atoms of two pyridine or nicotine molecules or the carbon atoms of two cyanide ions. The structure of dicyanide hemochromogen is shown in Fig. 7.

Carbonmonoxyhemoglobin and oxyhemoglobin have also been found to be diamagnetic [10] and hence to contain octahedral covalent bonds; in these molecules it is probable that four bonds are from iron to the four porphyrin nitrogen atoms, one to a nitrogen atom of the imidazole ring of a histidine residue of the globin,[11] and one to the attached carbon monoxide or oxygen molecule.

Octahedral covalent bonds have been reported from magnetic meas-

[9] Pauling and Coryell, Proc. Natl. Acad. Sci., U. S., 22, 159 (1936).
[10] Pauling and Coryell, ibid., 22, 210 (1936).
[11] Coryell and Pauling, J. Biol. Chem., 132, 769 (1940).

urements also for ferrihemoglobin cyanide, ferrihemoglobin hydrosulfide, ferrihemoglobin azide, imidazole-ferrihemoglobin, ammonia-ferrihemoglobin hydroxide, and ethylisocyanide-ferrohemoglobin, and essentially ionic bonds for ferrohemoglobin, ferrihemoglobin ion, ferrihemoglobin fluoride, ethanol-ferrihemoglobin ion, and ethanol-ferrihemoglobin hydroxide.[12] The magnetic data indicate the presence of bonds of intermediate type in ferrihemoglobin hydroxide.[12]
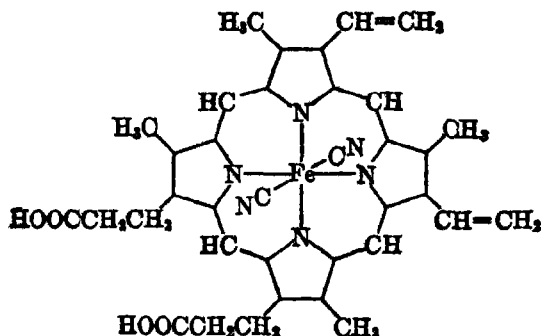
FIG. 7.—The structure of dicyanide hemochromogen, formed by combination of ferrous protoporphyrin and two cyanide ions. The octahedral orientation of six covalent bonds about the iron atom is indicated.

A complex of bivalent nickel in which the nickel atom forms four essentially covalent bonds directed toward the corners of a square of four atoms about the nickel atom and coplanar with it is diamagnetic; a nickelous complex with other types of bonds is paramagnetic. The observed diamagnetism of nickel protoporphyrin [9] shows that in this compound the four porphyrin nitrogen atoms are coplanar with the nickel atom and are attached to it by covalent bonds. A similar structure is shown for nickel phthalocyanine by its diamagnetism.

Multiple Bonds. The formal requirements for a double covalent bond between two atoms are the same as for two single covalent bonds; namely, the bond involves four electrons and four bond orbitals, two for each atom. A triple bond involves six electrons and six bond orbitals, three for each atom. Thus a first-row atom can form (with its four L orbitals) a maximum of four single covalent bonds, two single and one double, two double, or one single and one triple.

In writing electronic structures it is often convenient to use the customary valence-bond dashes, only the outer unshared electrons being represented by dots, as, for example,

[12] Coryell, Stitt, and Pauling, J. Am. Chem. Soc., **59**, 633 (1937); Coryell and Stitt, ibid., **62**, 2942 (1940); Russell and Pauling, Proc. Natl. Acad. Sci., U. S., **25**, 517 (1939).

$$H-H, \quad :\ddot{\underset{..}{C}}l-\ddot{\underset{..}{C}}l:, \quad H-\overset{\overset{\textstyle H}{|}}{\underset{\underset{\textstyle H}{|}}{C}}-H, \quad \overset{H}{\underset{H}{>}}C=C\overset{H}{\underset{H}{<}}, \quad H-C\equiv C-H, \quad H-\overset{\overset{\textstyle H}{|}}{\underset{\underset{\textstyle H}{|}}{C}}-\ddot{\underset{..}{O}}:$$

instead of

$$H:H, \quad :\ddot{\underset{..}{C}}l:\ddot{\underset{..}{C}}l:, \quad H:\overset{\overset{\textstyle H}{..}}{\underset{\underset{\textstyle H}{..}}{C}}:H, \quad \overset{H}{\underset{H}{.}}:C::C\overset{.H}{\underset{.H}{:}}, \quad H:C:::C:H, \quad H:\overset{\overset{\textstyle H}{..}}{\underset{\underset{\textstyle H}{..}}{C}}:\ddot{\underset{..}{O}}:$$
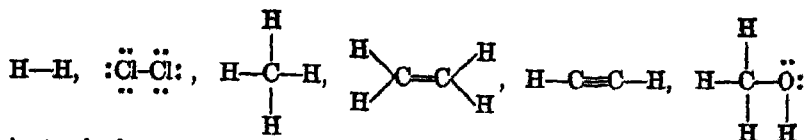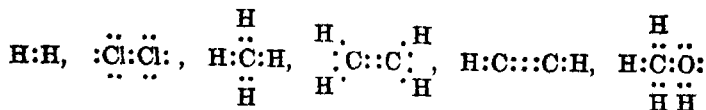
Moreover, it may also be convenient to represent the *formal charges* of the atoms by means of small plus and minus signs, the formal charge of an atom for a given electronic structure being calculated by assigning to that atom all its unshared electrons and one-half of the electrons which it shares with other atoms. Formal charges calculated in this way are shown in the following examples:

$$\left[\begin{array}{c} H \\ | \\ H-N-H \\ | \\ H \end{array}\right]^{+} \qquad \begin{array}{c} CH_3 \\ | \\ H_3C-\overset{+}{N}-\ddot{\underset{..}{O}}: \\ | \\ CH_3 \end{array} \qquad \begin{array}{c} H \\ | \\ H \searrow \overset{+}{N} \diagup \overset{-}{B} \diagdown \overset{+}{N} \diagup H \\ \| \quad \quad \| \\ H \diagup \overset{-}{B} \diagdown \overset{+}{N} \diagup \overset{-}{B} \diagdown H \\ | \\ H \end{array}$$
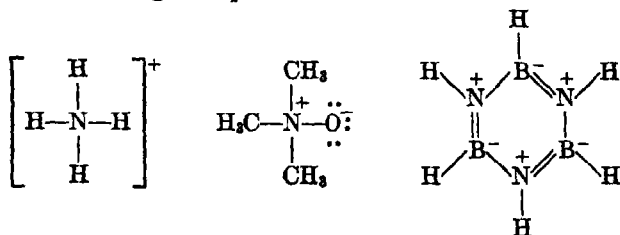
It must be recognized that these charges do not represent accurately the charge distribution for the molecule, inasmuch as such effects as polarization, partial ionic character of bonds, etc., are also of significance; but the formal charges are probably the expression of the most important effect.

In our discussion the terms *coördinate covalency* and *semi-polar double bonds* have not been used. The six single covalent bonds between C and Fe in $[Fe(CN)_6]^{=}$, for example, are sometimes called coördinate covalent bonds, on the basis of the supposition that this complex is formed from Fe^{+++} and 6 $(CN)^{-}$, the latter ions providing all the electrons for the bonds. These covalent bonds, once formed, do not differ in any way from other covalent bonds, however, and there seems to be no need for attempting to differentiate the C—Fe bond from the C—C bond in H_3C—CN, say, by the use of a different name. Similarly in trimethylamine oxide the bond between N and O is sometimes called a semi-polar double bond. This nomenclature may be convenient at times, the two atoms being actually held together by a single covalent bond and by an ionic bond (the electrostatic interaction of their formal charges); the use of a special symbol for the semi-polar double bond is unnecessary if the formal charges are shown in the structural formula.

The One-Electron Bond and the Three-Electron Bond.[13] The simplest molecules in which the one-electron bond and the three-electron bond occur are the hydrogen molecule ion, H_2^+, and the helium molecule ion, He_2^+, respectively. The hydrogen molecule ion consists of two protons (for each of which there is only one stable orbital, $1s$) and one electron. The two structures H· H^+ and H^+ ·H, in which the electron occupies first one and then the other $1s$ orbital, are equivalent, and so correspond to equal energy, satisfying the condition for resonance. The system may be expected to resonate between these two structures and thereby to be stabilized, forming a bond which we call the one-electron bond. This bond is only about one-half as strong as a shared-electron-pair bond, the dissociation energy of H_2^+ being 60,800 cal. per mole, as compared with 102,600 cal. per mole for H_2.

For He_2^+, with a $1s$ orbital for each nucleus and three electrons, there are also two equivalent structures, He: ·He^+ and He^+· :He, between which there is resonance, leading to the formation of a three-electron bond. This bond too is only about one-half as strong as a shared-electron-pair bond, the dissociation energy of He_2^+ being about 58,000 cal. per mole.

For the formation of a one-electron bond, an electron-pair bond, or a three-electron bond between two atoms there are needed two bond orbitals, one for each atom, and one, two, or three electrons, respectively. As mentioned above, a one-electron or three-electron bond is only about one-half as strong as an electron-pair bond. There is another difference in properties which causes the one-electron and three-electron bonds to be of only minor importance. An electron-pair bond can be formed between any two atoms, the conditions for resonance being always satisfied. On the other hand, the structures A· B and A ·B (or A: · B and A· :B) in general will not have approximately equal energy, and so will not satisfy the energy condition for resonance; only if A and B are atoms of the same element or are of such a nature as to cause the two structures to have nearly the same energy (as for two atoms adjacent in the periodic table) will resonance occur and a one-electron or three-electron bond be formed.

It is probable that the one-electron bond occurs in the boron hydrides,

$$\text{H H}$$
$$\cdot\cdot \;\; \cdot\cdot$$

B_2H_6 having the electronic structure H:B:B:H, in which two of the

$$\cdot \;\; \cdot$$
$$\text{H H}$$

boron-hydrogen bonds are one-electron bonds.[14]

[13] Pauling, *J. Am. Chem. Soc.*, **53**, 3225 (1931); ref. 1, Chapter VIII.

[14] Sidgwick, "The Electronic Theory of Valency," Oxford University Press, Oxford (1927), p. 103. For a more detailed discussion of the structure of the boron hydrides, see Penney and Pauling, *J. Am. Chem. Soc.*, **58**, 2403 (1936), and Bauer, *ibid.*, **59**, 1096 (1937).

The three-electron bond seems to occur in several molecules, between like atoms (as in $He_2{}^+$) or atoms which are adjacent to each other in the periodic table, and so are sufficiently alike to permit the resonance stabilizing this bond. Molecules and complexes containing this bond include NO, NO_2, $O_2{}^-$, O_2, SO, S_2, and ClO_2, to which are assigned the following structures, using $-$ and $=$ for the single and double covalent bonds, and . . . for the three-electron bond.

:N⋯O: Double bond plus a three-electron bond.

:O⁀N⋯O: Double bond to one oxygen atom, single bond plus a three-electron bond to the other.

$\left[\text{:O}\cdots\text{O:}\right]^-$ (Superoxide ion, as in KO_2.) Single bond plus a three-electron bond.

:O⋮⋮O:
:S⋮⋮O: Single bond plus two three-electron bonds (two unpaired electrons).
:S⋮⋮S:

:O⁀Cl⁀O: Single bond to one oxygen atom, single bond plus a three-electron bond to the other.

THE RESONANCE OF MOLECULES AMONG SEVERAL VALENCE-BOND STRUCTURES

The Meaning of Valence-Bond Formulas. By a formula containing the symbol C—C, representing a single covalent bond between two carbon atoms, it is meant that the bond between the two atoms is essentially the same as in ethane or in diamond; in quantum-mechanical language, the same wave function (or part of a wave function) would be used for the molecule under discussion as for ethane or diamond. Similarly C=C represents a bond as in ethylene, C≡C one as in acetylene, and so on.

[The symbol C=O is generally used to represent a double bond with about the same ionic character as in acetone. This ionic character arises in large part from resonance between the electronic structures

$$\begin{array}{c}R\\ \diagdown \\ \diagup\\ R\end{array}\!\!C\!=\!\ddot{O}\text{:} \quad \text{and} \quad \begin{array}{c}R\\ \diagdown \\ \diagup\\ R\end{array}\!\!\overset{+}{C}\!-\!\overset{..}{\underset{..}{O}}\text{:}^-$$
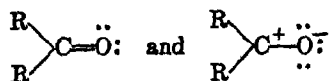
and it is sometimes (though not usually) convenient to give this resonance explicit discussion.]

It is found empirically that many molecules are of such a nature that for each a single valence-bond formula can be written which represents

the structure and properties of the molecule in a satisfactory way. Thus for these molecules the symbol C—C in the formula assigned means that the carbon–carbon bond has the chemical properties which have come to be associated with a single bond between two carbon atoms, that the internuclear distance of the two atoms is 1.54 Å, that the Hooke's-law force constant has the single-bond value, and so on.

The Structure of Simple Molecules. To many molecules, however, it is impossible to assign a single valence-bond structure which satisfactorily represents the molecule. Under these circumstances some new concepts and symbols might be introduced (for example, writing

 for benzene, without attempting to interpret this in terms of single

and double bonds). An alternative procedure, which has been found to be illuminating and practicable, is to assign to a molecule of this type more than one valence-bond structure, these structures all contributing to the normal state of the resonating molecule. In quantum-mechanical language, it is said that the wave function for the molecule is formed by linear combination of the wave functions corresponding to the valence-bond structures involved. The properties of the molecule are then those corresponding to the various valence-bond structures, cognizance being taken also of the extra stability resulting from the resonance itself.

As an example let us consider the nitro group in nitromethane or a

similar molecule. For this we write the formula , using all
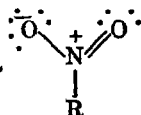
four L orbitals of nitrogen for bonds. However, there is another structure

which is entirely equivalent to this, namely, the structure ,
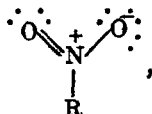
in which the two oxygen atoms have interchanged their roles. The two structures satisfy the conditions for resonance (they correspond to nearly the same nuclear configuration), and since they are equivalent they must contribute equally to the structure of the molecule. The molecule might then be represented by enclosing both formulas in brackets:
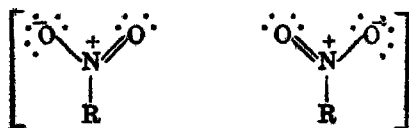
This is rather clumsy, however, and since it is evident that both equiva-

lent structures must be considered only one is usually written, it being understood that resonance with the other occurs.

It is to be emphasized again that in writing these two valence-bond structures for the molecule and saying that it resonates between them an effort is being made to extend the valence-bond picture to molecules to which it is not strictly applicable. This is not required but is chosen as a method in the hope of obtaining a satisfactory description of these unusual molecules, permitting the correlation (and "understanding") of the results of experiments on their chemical and physical properties and allowing predictions to be made in the same way as for molecules to which a single valence-bond structure can be assigned. The substance nitromethane is not a mixture of tautomeric molecules, some with one and some with the other of the two valence-bond structures written above. Instead, all the molecules have the same electronic structure, this being a structure which cannot be represented satisfactorily by any one valence-bond structure, but which is reasonably well represented by the two structures given above. The properties of the molecule are essentially those expected for an average of the two valence-bond structures, except for the stabilizing effect of the resonance energy. In nitromethane the two N—O bonds are equivalent. Each is a bond of a type intermediate between a double and a single bond; it is found experimentally that the properties of such a bond (interatomic distance, force constant) are determined mainly by the stronger of the bonds provided by the individual structures, the N—O bonds in the nitro group having properties close to those of a double bond.

It might well be asked by the chemist whether it is not then wise to write for nitromethane the valence-bond structure

$$O \diagdown \atop \diagup O$$
$$N$$
$$|$$
$$R$$
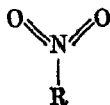
which gives a satisfactory representation of the properties of the N—O bonds. It does not seem wise to do this, for the following reasons. There are strong theoretical arguments showing that the maximum number of covalent bonds which a nitrogen atom (or other first-row atom) can form is four; the structure under discussion suggests that five covalent bonds can be formed. Moreover, the structure under discussion provides little stereochemical information—it could not be predicted whether the groups attached to the nitrogen atom are coplanar or not—whereas the resonating structure combined with the stereochemical knowledge of the tetrahedral nitrogen atom permits the conclusion that

the bonds are coplanar and that the O—N—O bond angle has approximately the single bond-double bond value 125° 16'.[*]

The assignment of a resonating structure to a molecule can sometimes be made on the basis of theoretical arguments, as in respect to the nitro group just discussed, for which the two reasonable valence-bond structures are equivalent. In general, such an assignment should be supported by experimental evidence, such as that provided by chemical properties, resonance energies (as obtained from thermochemical data, p. 1968), interatomic distances,[15] force constants of bonds obtained from Raman and infra-red spectra, dipole moments (p. 1720),[16] etc. If the reasonable valence-bond structures are not equivalent, knowledge of the magnitudes of the contributions of different structures to the actual structure of the molecule can be obtained from such data. Thus for carbon dioxide it is customary to write the structure $: \overset{..}{O} {=} C {=} \overset{..}{O} :$; however, the observed interatomic distances show definitely that the structures $: \overset{+}{O} {\equiv} C {-} \overset{-}{\underset{..}{O}} :$ and $: \overset{-}{\underset{..}{O}} {-} C {\equiv} \overset{+}{O} :$ contribute to just about as great an extent as the double-bonded structure, and this conclusion is supported by the resonance energy (the great thermodynamic stability of the molecule) and the force constants of the bonds. For nitrous oxide, on the other hand, the force constants [17] and interatomic distances [18] show resonance between the two structures $: \overset{-}{\underset{..}{N}} {=} \overset{+}{N} {=} \overset{..}{O} :$ and $: N {\equiv} \overset{+}{N} {-} \overset{-}{\underset{..}{O}} :$, the structure $: \overset{=}{\underset{..}{N}} {-} \overset{+}{N} {\equiv} \overset{+}{O} :$ not contributing.

On p. 1961 the structures $: \overset{.}{\underset{.}{O}} \diagdown_{N} \diagup \overset{.}{\underset{.}{O}} :$ and $: \overset{.}{\underset{.}{O}} \diagdown_{\underset{..}{Cl}} \diagup \overset{.}{\underset{.}{O}} :$ were assigned to NO_2 and ClO_2 respectively. Each of the molecules, of course, actually resonates between such a structure and the equivalent one in which the roles of the two oxygen atoms are interchanged.

For the carbon monoxide molecule the two structures $: C {=} \overset{..}{O} :$ and $: \overset{-}{C} {\equiv} \overset{+}{O} :$ have been suggested. Actually both of these contribute to the structure, which we write as $\{ : \overset{-}{C} {\equiv} \overset{+}{O} :, \ : C {=} \overset{..}{O} : \}$, the bond resonating between a double and triple covalent bond. The study of energy relations indicates that these structures are about equally important.

[*] This has been verified by experiment, the value 127° ± 3° being found: Brockway, and Pauling, J. Am. Chem. Soc., **57**, 2693 (1935).

[15] Pauling, Proc. Natl. Acad. Sci. U. S., **18**, 293 (1932).

[16] Sutton, Trans. Faraday Soc., **30**, 789 (1934).

[17] Plyler and Barker, Phys. Rev., **38**, 1827 (1931).

[18] Pauling, Proc. Natl. Acad. Sci. U. S., **18**, 498 (1932).

[19] See Pauling, J. Am. Chem. Soc., **54**, 988 (1932).

The carboxylic ions resonate between the two structures

which contribute equally because of their equivalence. In the carboxylic acids the two structures

are no longer equivalent, the second contributing less than the first, and the stabilizing effect of resonance being less than for the ion. This change in resonance energy, with the ion more stable than the acid, tends to assist in detaching the proton, and so gives these acids their great acid strength. The same result can be seen from another argument. The second of the structures written for the acid makes the oxygen atom to which the proton is attached positive in sign; it accordingly repels the proton, and so increases the acid strength.*

Single bond-double bond resonance also occurs in the carbonate ion, the nitrate ion, urea, guanidine, the acid amides, and similar compounds, the carbonate ion resonating among the following structures:
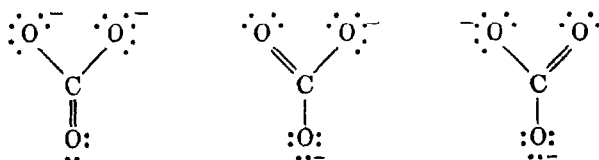
As another illustration of the application of the concept of resonance to the chemical properties of substances let us discuss the basic strengths of guanidine and substituted guanidines. The fact that guanidine is a strong base can be accounted for by either one of the two closely related arguments used above for the carboxylic acids. The guanidinium ion resonates among the three structures

* An interesting series of investigations of the effect of resonance on the acid strengths of substituted boric acids and other substances has been carried out by Branch and his collaborators: Yabroff, Branch, and Almquist, *J. Am. Chem. Soc.*, **55**, 2935 (1933); Branch, Yabroff, and Bettman, *ibid.* **56**, 937 (1934); Branch and Yabroff, *ibid.*, **56**, 2568 (1934).
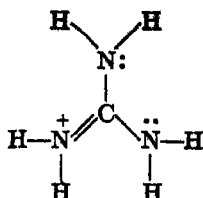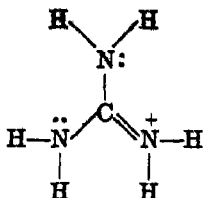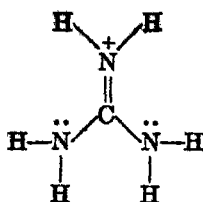
which are all equivalent, whereas guanidine itself resonates among the three structures
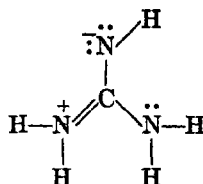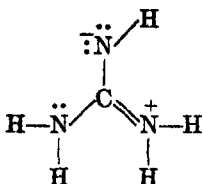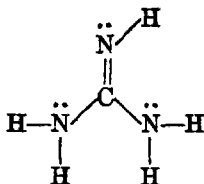
which are not equivalent, the first being more stable than the other two (it is the structure usually alone considered by the chemist) and hence contributing more to the actual state of the molecule, with resonance to the other two less important. In consequence the ion is stabilized by resonance more than the molecule, and the basic strength of the substance is increased by resonance.

It may be predicted that monoalkyl-substituted and N,N-dialkyl-substituted guanidines are weaker bases than guanidine itself, for the following reason. The replacement of one or two hydrogens of an —NH$_2$ group by alkyl radicals tends to prevent the double bond from swinging to this group, because carbon is more electronegative than hydrogen and hence tends to cause the adjacent nitrogen atom not to assume a positive charge. In consequence resonance of the double bond is to some extent restricted to the two other nitrogen atoms. This causes a decrease in the basic strength towards that characteristic of an imidine, the decrease

being about twice as great for HNC⟨$^{NH_2}_{NR_2}$ as for HNC⟨$^{NH_2}_{NHR}$. A very

much larger effect is expected for the N,N′-dialkylguanidines. The alkyl groups on two of the nitrogen atoms would tend to force the

double bond to the third nitrogen atom, the structure R—N⟨C⟩N—R
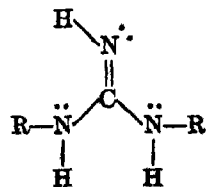
being more important than the other two. This nitrogen atom would hence have little tendency to add a proton, and the substance would be a weak base. The tetraalkylguanidines $HNC{\overset{NR_2}{\underset{NR_2}{\diagdown}}}$ would be still weaker bases, approaching the non-resonating imines still more closely. On the other hand, the N,N',N''-trialkylguanidines may be expected to be about as strong bases as guanidine itself, inasmuch as the conditions for resonance in this molecule and its symmetrical ion are the same as for guanidine itself and its ion. These various conclusions are in agreement with the available experimental data;[20] guanidine, the monoalkylguanidines, N,N-dimethylguanidine, and N,N',N''-trimethylguanidines are strong bases, whereas the N,N'-dialkylguanidines are weak.

Empirical Resonance Energies. Thermochemists have often attempted to assign constant energy values to the bonds in molecules in such a way that the total energy of formation of a molecule from separated atoms could be expressed as a sum of bond energies. It is found that by restricting the discussion to molecules to each of which a single valence-bond structure can be confidently assigned this program can be carried out with considerable success; a table of bond energies can be constructed with which energies of formation of non-resonating molecules reliable to a few thousand calories can be calculated.

On applying this table to resonating molecules it is found that *the actual energy of formation of the molecule invariably is greater than the calculated value*; that is, the molecule is actually more stable than it would be if it had the valence-bond structure assumed for it in making the bond-energy calculation.* This result is the one required by the quantum mechanics, according to which resonance always exerts a stabilizing action on the molecule. The difference between the observed energy of formation (obtained from heats of combustion or other thermochemical data) and the value calculated by bond energies for an assumed valence-bond structure is an *empirical value of the resonance energy* of the mole-

[20] Davis and Elderfield, *ibid.*, **54**, 1499 (1932).

* See Pauling and Sherman, *J. Chem. Phys.*, **1**, 606 (1933), for the details of this treatment. In calculating resonance energies it is for convenience only that the thermochemical data are converted into energies of formation of molecules from separated atoms; the same results can be obtained by dealing directly with heats of formation from elementary substances in their standard states or with heats of hydrogenation reactions or other reactions. Many important results regarding resonance energies in unsaturated and aromatic compounds have been obtained recently by Kistiakowsky and his collaborators by the direct measurement of heats of hydrogenation [Kistiakowsky, Ruhoff, Smith, and Vaughan, *J. Am. Chem. Soc.*, **58**, 137, 146 (1936)]. The values found in this way are in general agreement with the less accurate values, obtained from heats of combustion, given in Table I, the values of the resonance energy found in these two ways for benzene, for example, being 36,000 and 39,400 cal. per mole, respectively.

cule, which resonates between the structure assumed and other structures.
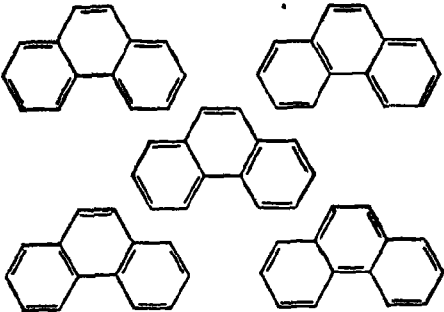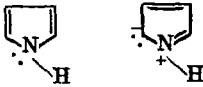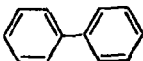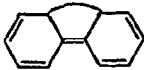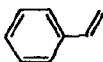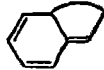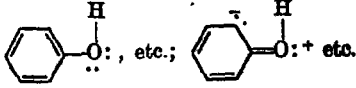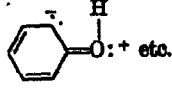
Some empirical resonance energy values are given in Table I. It is seen that the values support the statements made in the preceding section regarding the structure of some simple molecules. For carbon mon-

TABLE I

EMPIRICAL VALUES OF RESONANCE ENERGY

Substance	Resonance energy (in calories per mole)	The principal resonating structures*
CO	58,000	:C=Ö: , ⁻:C≡O:
CO_2	31,600	:Ö=C=Ö: , :O≡C—Ö: , :Ö—C≡O:
SCO	19,400	Same as for CO_2
CS_2	10,600	Same as for CO_2
RNCO (R=CH₃,C₂H₅)	6,600	R—N=C=Ö: , R—N≡C—Ö: , R—N—C≡O:
RCO_2H	27,600	
$RCONH_2$	21,000	
$CO(NH_2)_2$	36,600	
R_2CO_3	41,600	Same as for urea
Benzene	39,400	
Naphthalene	74,600	
Acenaphthene	71,000	Same as for naphthalene
Anthracene	104,700	

* In each case the resonance energy is calculated relative to the first of the structures given.

TABLE I—*Continued*

Substance	*Resonance energy* (in calories per mole)	*The principal resonating structures*
Phenanthrene	110,000	

Pyridine	43,100	Same as benzene
Quinoline	69,400	Same as naphthalene
Pyrrole	22,600	

Furan	23,000	Same as pyrrole
Thiophene	31,000	Same as pyrrole
Indole	54,000	Similar to pyrrole
Carbazole	91,000	Similar to pyrrole
Biphenyl	8,000†	

| 1,3,5-Triphenylbenzene | 25,000† | Similar to biphenyl |
| Phenylethylene | 6,700† | |

Stilbene	15,400†	Similar to phenylethylene
Phenylacetylene	10,400†	" " "
Benzonitrile	4,900†	" " "
Benzoic acid	4,200†	" " "
Benzaldehyde	3,500†	" " "
Acetophenone	7,100†	" " "
Benzophenone	10,400†	" " "
Phenol	6,700†	

| Aniline | 6,000† | Same as phenol |

† Extra resonance energy, not including benzene resonance.

oxide, resonance with the structure $:C\!\!\equiv\!\!\overset{+}{O}:$ stabilizes the molecule to the extent of 58,000 cal. per mole relative to the structure $:C\!\!=\!\!\ddot{O}:$; if this resonance did not occur the substance would not be thermodynamically stable. The observed resonance energy of 31,600 cal. per mole for carbon dioxide shows that the structure $:\ddot{O}\!\!=\!\!C\!\!=\!\!\ddot{O}:$ for this molecule does not alone represent the molecule satisfactorily. The most reasonable structures to provide this resonance energy are $:O\!\!\equiv\!\!\overset{+}{C}\!\!-\!\!\overset{..}{\underset{..}{O}}\overset{-}{:}$ and $\overset{..}{\underset{..}{:}}\overset{+}{O}\!\!-\!\!C\!\!\equiv\!\!O:$, and as mentioned above, it has been verified by arguments based on interatomic distances that these structures are almost as important as the first for this molecule. Resonance of this type is much less important in carbon disulfide.[21]

In the carboxylic acids and the acid amides the resonance of the double bond between two positions gives rise to a resonance energy of about 25,000 cal. per mole; and in urea and the esters of carbonic acid resonance of the double bond among three positions leads to a resonance energy of about 40,000 cal. per mole, a reasonable value in comparison with the foregoing one.

The remaining values in the table will be discussed in later sections.

THE STRUCTURE OF BENZENE AND OTHER AROMATIC MOLECULES

The Structure of Benzene (p. 119). The benzene molecule is known from electron and x-ray diffraction studies to be a plane, the six carbon atoms lying at the corners of a regular hexagon 1.39 Å on edge. This nuclear configuration is compatible with the Kekulé structures,

and , which the chemist immediately writes as the most reasonable. These two structures do indeed make the largest contributions to the structure of the normal benzene molecule. The detailed investigation * of the problem has shown that the resonance between the two Kekulé structures stabilizes the molecule to the extent of about 31,000 cal. per mole; in addition the less stable structures† C, D, and E make a

[21] Cross and Brockway, *J. Chem. Phys.*, **3**, 821 (1935).

* Of the two general quantum-mechanical methods which have been applied to this problem, the molecular orbital method and the valence-bond method, only the latter which is the more closely related to the usual concepts of chemistry will be discussed. See Hückel, *Z. Phys.*, **70**, 204; **72**, 310 (1931); **76**, 628 (1932); Pauling and Wheland, *J. Chem. Phys.*, **1**, 362 (1933); ref. 1, Chapter IV.

† It is convenient to call the valence-bond structures with the maximum number of double bonds *unexcited structures*, and those with a smaller number (the less important ones) *excited structures*.

small contribution, increasing the resonance energy to about 39,000 cal. per mole. This resonance energy makes the benzene ring about 39,000 cal. per mole more stable than a ring with three non-interacting double bonds, and gives it its peculiar aromatic properties.

As a result of resonance, each of the carbon-carbon bonds assumes properties similar to those of a double bond (except for the greater stability conferred by the resonance energy). Hence all the atoms in the molecule are restricted to one plane (as in ethylene, for example), and the bond angles are restricted to values near the tetrahedral angles 109° 28' and 125° 16'. These conditions are satisfied by the six-membered ring of benzene. On the other hand, the angles of 90° and 135° in plane rings of cyclobutadiene and cyclooctatetraene, respectively, introduce so much strain as to counteract the stabilizing effect of resonance.[22]

The quantum-mechanical treatment of benzene has been found to provide an explanation of many characteristic properties of the substance. On p. 1975 directed substitution is briefly discussed from this viewpoint. The effect of five-membered and six-membered saturated side rings in influencing the properties of benzene, discovered by Mills and Nixon,[23] has been shown [24] to depend on a change of a few per cent in the contributions of the two Kekulé structures to the resonating structure of the molecule.

Naphthalene, Anthracene, Phenanthrene. The three most important valence-bond structures for naphthalene are the following:
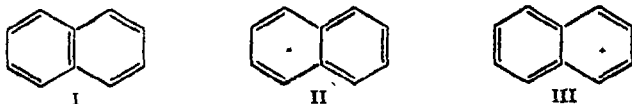
These contribute about equally to the resonating structure, the symmetrical structure I being somewhat more important than II and III. In addition smaller contributions are made by various less stable structures. This resonance stabilizes the molecule by 74,000 cal. per mole, giving naphthalene aromatic properties similar to those of benzene.[25] As in benzene, each carbon-carbon bond has properties approaching those for

[22] Penney, *Proc. Roy. Soc. (London)*, **A146**, 223 (1934).
[23] Mills and Nixon, *J. Chem. Soc.*, 2510 (1930).
[24] Sutton and Pauling, *Trans. Faraday Soc.*, **31**, 939 (1935).
[25] Pauling and Wheland, *J. Chem. Phys.*, **1**, 362 (1933); Sherman, *ibid.*, **2**, 488 (1934).

a double bond; the entire molecule is planar,[20] with bond angles near 120°.

In anthracene four structures
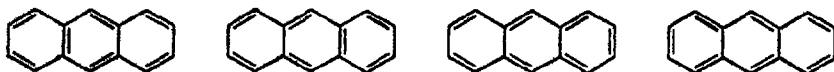
make the largest contributions to the normal state of the molecule.

Five structures are important for phenanthrene:
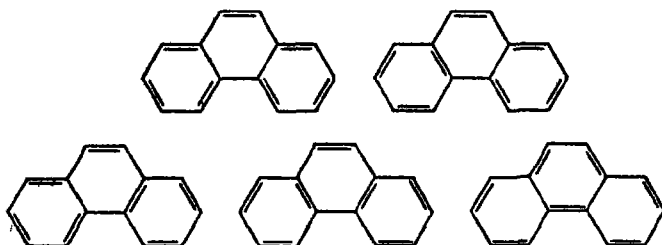
The resonance energies for these two molecules are 104,700 cal. per mole and 110,000 cal. per mole, respectively, both substances thus having aromatic properties, with phenanthrene about 5000 cal. per mole more stable than anthracene (Table I). The additional stability of phenanthrene is attributed to the fact that it resonates among five unexcited structures rather than four. It is found that in larger polycyclic molecules also greater stability accompanies more extensive branching, which increases the number of unexcited structures contributing to the resonance energy.

It must be emphasized that the larger resonance energy of naphthalene than of benzene does not require naphthalene to be more aromatic than benzene in its chemical reactions, inasmuch as the stabilizing action of the resonance energy is divided among five double bonds in the former and only three in the latter molecule. In general it is necessary to consider the resonance energy of the products of reaction also in discussing chemical properties of resonating molecules. Thus on hydrogenating benzene to 1,3-cyclohexadiene (with about 6000 cal. per mole resonance energy of conjugation of the two double bonds) there is a loss of resonance energy of about 33,400 cal. per mole, whereas the loss on hydrogenation of naphthalene in the 1,2-positions is only 29,200 (assuming 6000 cal. per mole energy of conjugation of double bond and benzene ring in 1,2-dihydronaphthalene). In consequence naphthalene may be expected to be more easily hydrogenated than benzene.* A very simple treatment

[20] Robertson, *Proc. Roy. Soc.* (*London*), **A142**, 674 (1933).

* For a more detailed treatment of hydrogenation, see Pauling and Sherman, *J. Chem Phys.*, **1**, 679 (1933).

of bond character in aromatic hydrocarbons which leads to conclusions in general agreement with the known chemical properties of the substances can be made on the basis of the unexcited structures of the molecules. By examining the unexcited structures of an aromatic hydrocarbon we may assign to each bond a fraction representing its double-bond character, this fraction being the ratio of the number of structures placing a double bond in this position to the total number of structures. This gives the following results:

Benzene   Naphthalene

Anthracene   Phenanthrene
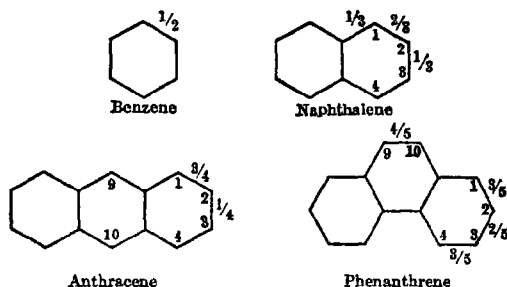
In benzene each bond has one-half double-bond character, whereas in naphthalene the 1,2-bonds have two-thirds and the 2,3-bonds one-third double-bond character. These numbers cannot be given a simple quantitative interpretation in terms of chemical reactivity; they do demand, however, that qualitative relations be satisfied. The 1,2-bonds in naphthalene must be much closer to ordinary double bonds in their properties than are the benzene bonds, which in turn are much more like double bonds than are the 2,3-bonds in naphthalene, the last, indeed, having practically no properties of a double bond. These statements are in agreement with general chemical experience. Various coupling reactions of naphthalene involving the 1,2-positions show the 1,2-bonds to have, to a pronounced extent, the properties of a double bond, whereas the 2,3-bonds show no such properties.[27]

The 1,2-bonds in anthracene have a still more pronounced double-bond character,[28] which in turn is exceeded by that for the 9,10-bond in phenanthrene. This explains the fact that phenanthrene (despite its greater thermodynamic stability than anthracene, consequent to its greater resonance energy) is more reactive than anthracene.

The activity of anthracene in the 9,10-positions cannot be discussed in this way. Instead we may consider the fraction of the number of unexcited structures for the product of an addition reaction at these

[27] Fieser and Lothrop, ibid., 57, 1459 (1935), and earlier references quoted by them.
[28] Fieser and Lothrop, ibid., 58, 749 (1936), and references quoted by them.

positions in comparison with other positions. For the 9,10-positions there
are four,
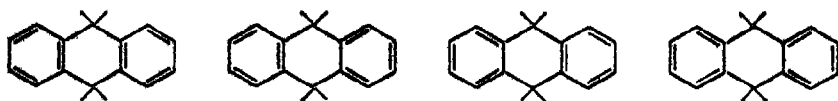
for the 1,2-positions three,
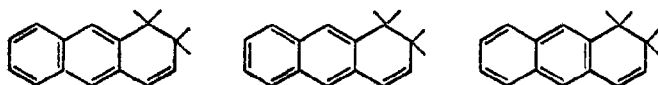
and for the 2,3-positions only one,
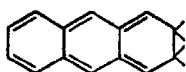
A large reactivity of anthracene toward addition reactions (and other
reactions which involve these as intermediates) in the 9,10-positions is
accordingly expected; smaller reactivity in the 1,2-positions; and negli-
gible reactivity in the 2,3-positions. This is in agreement with experi-
ment.

Heterocyclic Molecules. The resonance energies of pyridine and
quinoline are about the same as for benzene and naphthalene, respec-
tively, the same valence-bond structures contributing as for these mole-
cules, and the aromatic character consequently being just as pronounced.

In pyrrole, furan, and thiophene, with resonance energies in the
neighborhood of 20,000–30,000 cal. per mole, the structures other than
which make the largest contribution are of the types
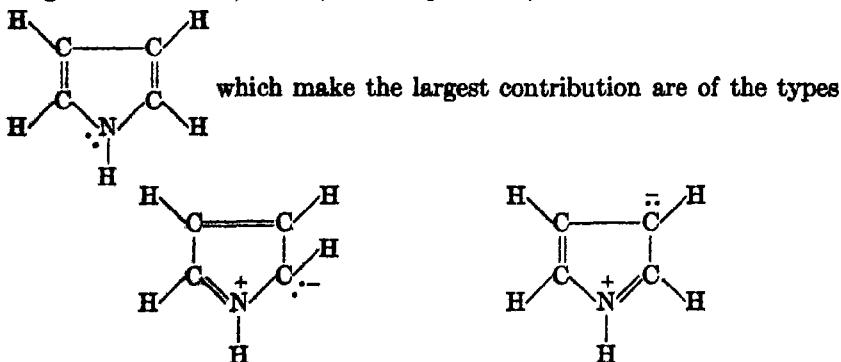
the unshared electron pair resonating among all the atoms of the ring.
Similar structures also contribute in indole, carbazole, and other hetero-
cyclic compounds, giving rise to resonance energies of about the same
magnitude as for aromatic hydrocarbons with the same number of rings.

Orientation of Substituents in Aromatic Molecules.* When a substituent is introduced into an aromatic molecule it may enter into certain of the available positions more readily than into others. This phenomenon has been exhaustively studied, and empirical rules have been found which describe the experimental results fairly satisfactorily (p. 174). Thus in a monosubstituted benzene C_6H_5R the groups R = CH_3, F, Cl, Br, I, OH, NH_2, etc., are *ortho-para* directing, and R = CO_2H, CHO, NO_2, $N(CH_3)_3^+$, etc., are *meta* directing. Most *ortho-para* directing groups activate the molecule so that substitution takes place more readily than in benzene itself, and most *meta* directing groups have a deactivating effect. In naphthalene, substitution occurs at the α-position, in furan, thiophene, and pyrrole at the α-position, and in pyridine at the β-position, all these molecules except pyridine being more active than benzene.

During the last fifteen years a qualitative theory has been developed † which accounts satisfactorily for the phenomenon in its major features, and recently a quantitative treatment based on quantum mechanics has been carried out,[29] with a degree of success which provides strong support for the theory.

The theory is based on the consideration of the distribution of electric charge (the electron distribution) in the molecule in which substitution is taking place. In a benzene molecule the six carbon atoms are equivalent, and the charge distribution is accordingly such as not to make one carbon atom different from another. In the molecule C_6H_5R, with R attached to carbon atom 1, the electron distribution will in general be affected by the group R in such a way as to change the charges on the *ortho* (2 and 6), *meta* (3 and 5), and *para* carbon atoms. Moreover, the electron distribution may also be changed somewhat on the approach of the substituting group R' to one of the carbon atoms ("polarization" of the molecule by the group R'); in benzene the polarization of one carbon atom by the group would be the same as for another, but in a substituted benzene the polarization would in general vary from atom to atom, and so might cause a difference in behavior of different positions. The fundamental postulate of the theory of orientation of substituents is the following: *In an aromatic molecule undergoing substitution by the group R', the rate of substitution of R' for hydrogen on*

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* The discussion in this section refers to substitution reactions involving the more common (cationoid) reagents.

† Many workers, including Fry, Stieglitz, Lapworth, Lewis, Lucas, Lowry, Robinson, and Ingold, have contributed to the theory. For an excellent review see Ingold, *Chem. Rev.*, **15**, 225 (1934).

[29] Wheland and Pauling, *J. Am. Chem. Soc.*, **57**, 2086 (1935); see also Ri and Eyring, *J. Chem. Phys.*, **8**, 433 (1940).

the ith carbon atom increases with increase in the negative charge on the ith carbon atom when the group R' approaches it.

Substitution is thus assumed to take place preferentially on that carbon atom on which the negative charge is the largest. There are two principal ways in which the charge distribution can be affected by the group R, for each of which it has been assumed, and has been verified by quantum-mechanical calculations,* that the *ortho* and *para* carbon atoms are about equally affected, the *meta* carbon atoms being affected to a much smaller extent.

The first effect of the group R, called the *inductive effect*, results whenever the electron affinity of the group is larger than or smaller than that of hydrogen. In the former case electrons are attracted to the group and to the attached carbon atom 1; calculation shows that they are removed mainly from the *ortho* and *para* carbon atoms.† Consequently the rate of substitution at the *ortho* and *para* positions will be greatly decreased and that at the *meta* positions somewhat decreased; the group R will be *meta* directing, with deactivation. An example of such a group is $N^+(CH_3)_3$, in trimethylphenylammonium ion; the nitrogen atom has a larger electron affinity than hydrogen, and its attraction for electrons is further intensified in this case by its positive charge. The same effect is seen in pyridine; the nitrogen atom attracts electrons mainly from the α- and γ-carbon atoms, and consequently pyridine substitutes in the β-positions, and is less active than benzene. Toluene shows the opposite effect. Electric moment measurements show that the methyl group loses electrons to the ring; these go mainly to the *ortho* and *para* carbon atoms, which are thus activated, toluene substituting in these positions, and substitution occurring with greater ease than in benzene.

It might be expected that F, Cl, Br, I, OH, and NH_2 would be *meta* directing, inasmuch as these groups all have larger electron affinities than hydrogen. Actually they are all *ortho-para* directing, the inductive

---

* Wheland and Pauling, *loc. cit.* This was first shown, for the inductive effect alone, by Hückel, *Z. Physik,* **72,** 310 (1931).

† This result can be seen from the following qualitative argument. An excess negative charge attracted to carbon atom 1 is accounted for by resonance to ionic structures in which this atom has an unshared pair. There are only three unexcited ionic structures of this type,
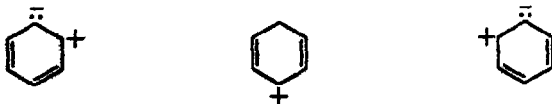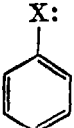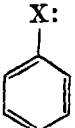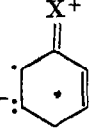
and they correspond to removing electrons equally from the two *ortho* atoms and the *para* atom. The *meta* atoms remain unaffected so long as the excited ionic structures are not considered: their effect would be small.

effect for them being overcome by another effect, called the *resonance effect* (or sometimes the *tautomeric* or *electromeric effect*, p. 1845). For the molecules considered in the preceding paragraph a consideration of resonance was not needed, except to the same extent as in benzene itself. If the group R possesses an unshared pair of electrons, however, other structures make an appreciable contribution to the normal molecule.*
Thus the structures
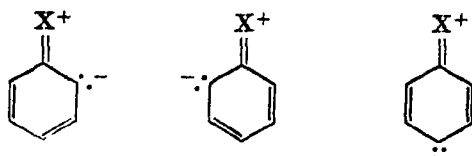
in which the unshared pair resonates to the *ortho* and *para* positions are

almost as stable as ... and ... , each possessing three double

bonds.   (Excited structures such as: ... , with two electrons not

involved in a bond between adjacent atoms, are much less stable and need not be considered.)   These three additional structures increase the electron density on the *ortho* and *para* atoms, and so make the groups *ortho-para* directing, the resonance effect being more significant than the inductive effect.
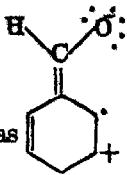
In benzaldehyde and many similar molecules, on the other hand, resonance directs toward the *meta* positions, this effect of resonance resulting whenever the substituted group R contains an electronegative atom and a double bond conjugated with the benzene ring † (R = CO_2H, CHO, NO_2, $COCH_3$, CN, etc.).   The structures leading to this effect are of the type
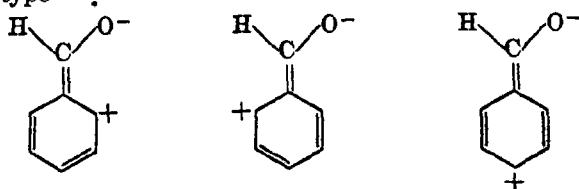
* The contribution of these structures to the resonance energy amounts to about 6000 cal. per mole (Table I, phenol and aniline).

† The resonance energy of this conjugation amounts to 5000 to 10,000 cal. per mole (Table I).

which decrease the electron density in the *ortho* and *para* positions, thus permitting reaction at the *meta* positions. (The contribution of excited

structures such as is small because of their instability; it suffices

to produce some deactivation, however.)

So far only the permanent charge distribution, as influenced by the inductive and resonance effects, has been discussed. The discussion for a monosubstituted benzene can be summarized as follows. When resonance does not occur, substitution is usually determined by the inductive effect, an electron-attracting group $(N^+(CH_3)_3)$ being *meta* directing and an electron-repelling group (CH_3) *ortho-para* directing. The resonance effect, which when present is usually more powerful than the inductive effect, is *meta* directing when the group contains a double bond conjugated with the benzene ring, and *ortho-para* directing when the group contains an unshared electron pair on the atom adjacent to the benzene ring.

In a few cases (naphthalene, for example) it is necessary to consider also the polarization of the molecule by the attacking group; as yet no general qualitative rules have been formulated regarding this effect, though some quantitative calculations have been carried out. The effect can be treated qualitatively by the consideration of the number of unexcited ionic structures placing an unshared pair on the carbon atom being attacked. For the α-position of naphthalene there are seven:
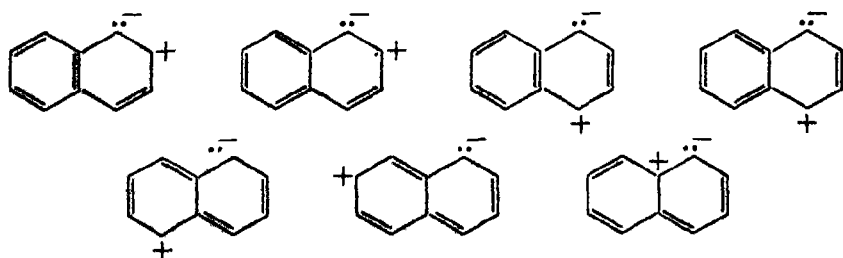
and for the β-position only six:
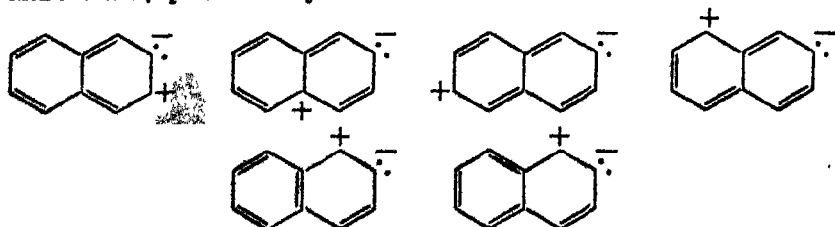
hence the polarization by the attacking group will be greater for the *o*-position, and substitution will take place there. The same argument can be applied to phenylethylene, which is *ortho-para* directing. Its zero electric moment shows that the vinyl group has no pronounced difference in electron affinity from hydrogen, and so will produce no inductive effect, and calculation shows that there is no resonance effect (which depends on the presence of an electronegative atom as well as of the double bond). However, polarization is greater in the *ortho* and *para* positions than in the *meta* positions, the former having four unexcited ionic structures, such as the following for *ortho*:
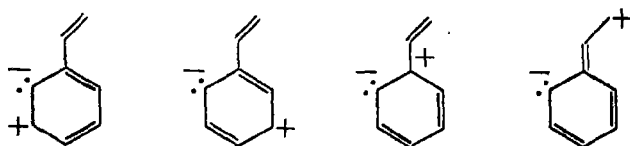
and the latter only three:
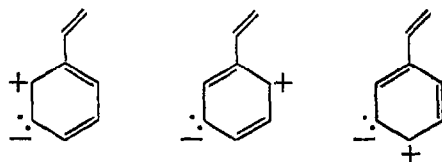
The Hydrocarbon Free Radicals (p. 581). The modern theory of the stability of the aromatic free radicals is based on resonance.* Increase in the degree of dissociation of a substituted ethane, R_3C—CR_3, might result either from a decrease in stability of the undissociated molecule or an increase in stability of the products of dissociation, the free radicals R_3C. For the hexaalkylethanes, which do not dissociate easily,

the electronic structure is written, and for the corresponding free radical the structure , the odd electron (free valence) being located on the methyl carbon atom. The introduction of an aryl group as a substituent R, however, provides additional structures for the radical; it is principally the energy of resonance among these which stabilizes the free radical and increases the degree of dissociation of the substituted ethane.

* The idea was developed empirically by Burton and Ingold, *Proc. Leeds Phil. Lit. Soc. Sci. Sect.*, **1**, 421 (1929); Ingold, *Ann. Repts. Chem. Soc. (London)*, **25**, 154 (1928), and was put on a quantitative basis by the quantum-mechanical calculations of Pauling and Wheland, *J. Chem. Phys.*, **1**, 362 (1933), and Hückel, *Z. Physik*, **83**, 632 (1933).

For simplicity, the molecule $C_6H_5CH_2$—$CH_2C_6H_5$, *sym*-diphenylethane, may be considered, and the discussion of resonance may be restricted to the structures with the greatest stability (those with the maximum number of double bonds). For the undissociated molecule there is resonance among the four Kekulé structures,
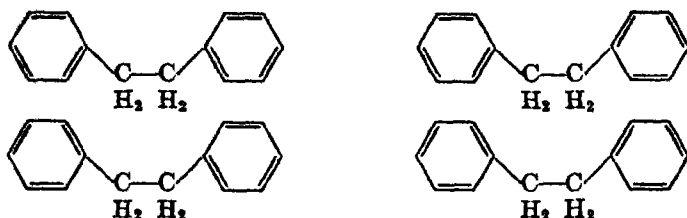
whereas each of the free radicals can resonate among the five structures:
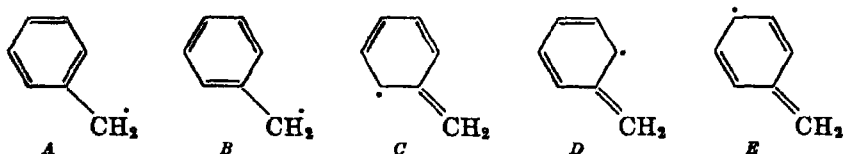
If the radical were restricted to resonance between the Kekulé structures *A* and *B*, with the free valence on the methyl carbon, resonance would stabilize the radicals to just the same extent as the undissociated molecule, which would then have only the same tendency to dissociate as a hexaalkylethane. But actually the five structures *A*, *B*, *C*, *D*, and *E* (each with three double bonds) contribute about equally to the structure of the radical, which thus resonates among five structures (instead of two), and is accordingly stabilized by the additional resonance energy, which is found on calculation to be about 15,000 cal. per mole.

The effect of two phenylmethyl radicals in decreasing the energy of dissociation by about 30,000 cal. per mole is not large enough to cause dissociation to an appreciable extent, inasmuch as the energy required to break the carbon-carbon bond in ethane is of the order of magnitude of 85,000 cal. per mole. In triphenylmethyl, however, the odd electron can resonate among nine positions (the *ortho* and *para* positions of the three phenyl groups) in addition to that on the methyl carbon atom; this leads to an additional resonance energy of about 38,000 cal. per mole, so that two such radicals stabilize the system by an amount (76,000 cal. per mole) sufficient to decrease the dissociation energy to only a few thousand calories per mole, resulting in extensive dissociation. In tribiphenylmethyl, in which the odd electron resonates among nineteen positions, the dissociating effect is still larger, the additional resonance energy being about 44,000 cal. per mole.

The quantum-mechanical discussion has been carried out by two distinct methods, the results of which are in essential concordance.[30] The detailed agreement with experiment in regard to such fine points as the greater dissociating action for α- than for β-naphthyl and for two phenyls than for fluoryl leaves little doubt that resonance of the odd electron (free valence) among several positions in the radical is the principal influence stabilizing the free radicals. It is also probable that other factors, such as the steric effects of the large groups, have a considerable influence in increasing the degree of dissociation.*

The positive and negative free radical ions have about the same possibilities of resonance as the free radicals themselves, the positive or negative charge (the latter being an unshared pair of electrons) resonating among the same positions as the odd electron; so that for all free radicals about the same values of the ionization potential and the electron affinity may be expected.[31] This conclusion is in agreement with the experimental results obtained by Bent,[32] who has found values of about 60,000 cal. per mole for the electron affinity of several different free radicals.

The Color of Dyes.† It has been gradually recognized that the intense color of the triphenylmethane dyes and of other dyes whose constitution is well understood is closely related to resonance. Baeyer [33] suggested that in p,p'-diaminotriphenylcarbinol hydrochloride (Döbner's violet), for example, the color is due to the oscillation of a chlorine atom from one end of the molecule to the other.
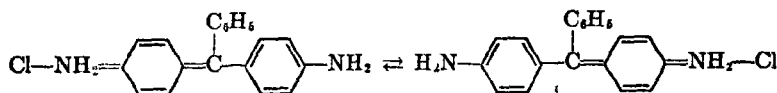
With the recognition of the ionic character of the bond to chlorine, this suggestion was revised by Adams and Rosenstein,[34] who correlated the color with an oscillation of an electron:
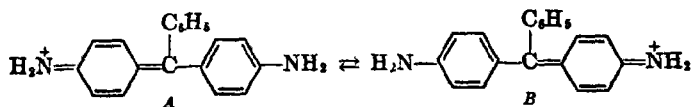
[30] Wheland, *J. Chem. Phys.*, **2**, 474 (1934).

* For a discussion of these points see Wheland, *ibid.*, **2**, 474 (1934); Bent and Ebers, *J. Am. Chem. Soc.*, **57**, 1242 (1935); and Preckel and Selwood, *ibid.*, **63**, 3397 (1941).

[31] Wheland, *loc. cit.*; Pauling and Wheland, *J. Chem. Phys.*, **3**, 315 (1935); Hylleraas, *ibid.*, **3**, 313 (1935).

[32] Bent, *J. Am. Chem. Soc.*, **52**, 1498 (1930); **53**, 1786 (1931).

† Bury, *ibid.*, **57**, 2115 (1935). We have extended Bury's discussion with the argument given in the second paragraph.

[33] Baeyer, *Ann.*, **354**, 152 (1907).

[34] Adams and Rosenstein, *J. Am. Chem. Soc.*, **36**, 1472 (1914).

From the modern point of view intense color is the result of a transition of the molecule from its normal electronic state to an excited electronic state, with absorption of light, the transition having a very high probability if the electric-moment matrix element associated with it is large. Now the two valence-bond structures A and B represented above are equivalent; hence neither one represents the normal state of the molecule, which instead is represented by a combination of the two. There is also another combination of the same structures which represents an excited state of the molecule. Now it can be shown that the electric-moment matrix element associated with transition between these two states is very large, by the following argument. The negative ion may be considered to be near the center of the molecule.* Then structure A corresponds to a very large dipole moment (p. 1752) in one direction and B to the same moment in the other direction. The actual molecule in its normal state and the excited state under consideration will have zero moment, however, because with equal resonance between A and B their moments neutralize each other. It can be shown by quantum-mechanical methods that under these circumstances the electric-moment matrix element associated with the transition between the normal and the excited resonating state has the same magnitude as the moment associated with structures A and B, and is hence in this case very large; consequently the substance is very deeply colored, with the absorption of light corresponding to this electronic transition. The results of this argument can be summarized by saying that intense color results from resonance between two equivalent or nearly equivalent structures with which a large dipole moment is associated (the actual electronic transition being between resonating structures formed from these).

Nitrogen and oxygen atoms are important in dyes in order to introduce the large electric moments. The structures which by resonance give the normal and significant excited states for some dyes are listed below.

The anion of benzaurin:
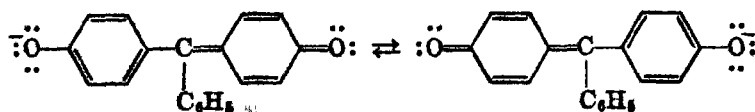
* This assumption is not necessary, but simplifies the argument.

The cation of acridine orange:
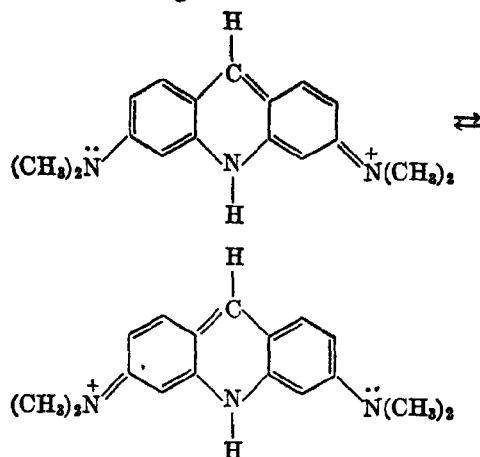
The dimethylpyronium cation:
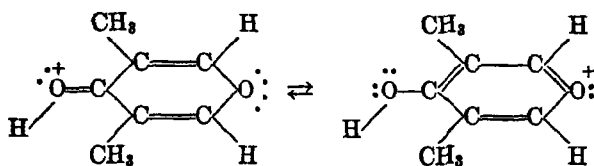
The development of a modern theory of the color of dyes is progressing rapidly at present; for information the reader is referred to recent papers.[35, 36]

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[35] Pauling, *Proc. Natl. Acad. Sci. U. S.*, **25**, 577 (1939).

[36] Lewis and Calvin, *Chem. Rev.*, **25**, 273 (1939); Förster, *Angew. Chem.*, **52**, 223 (1939); Sklar, *J. Chem. Phys.*, **7**, 984 (1939); Brooker, Sprague, Smyth, and Lewis, *J. Am. Chem. Soc.*, **62**, 1116 (1940); Granick, Michaelis, and Schubert, *ibid.*, **62**, 1802 (1940).

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<u>Part II:</u>

Paper No.	Title of Paper	Maximum Marks	Marks Obtained
VI (Main)	Microeconomics	50	47
VII (Main)	Macroeconomics	50	42
VIII (Main)	Economic History of India (1857-1947)	50	36
IX (Main)	India's Economic Development since 1947	100	61
X (Subsidiary)	Linear Algebra and Calculus	50	42
XI (Subsidiary)	Symbolic Logic	50	45
	Total	350	273

<u>Part III:</u>

Paper No.	Title of Paper	Maximum Marks	Marks Obtained
XII (Main)	Economy, State and Society	50	
XIII (Main)	Development Theory and Experience	100	
XIV (Main)	Topics in Microeconomics	50	
XV (Main)	Topics in Macroeconomics	50	
XVI (Main)	Introductory Econometrics	50	
XVII (Main)	International Economics	50	
	Total	350	

Over the **three-year degree** course, **Skand Goel** has obtained an aggregate of **73.69 %** and secured a **First Division***. The medium of instruction has been **English** throughout.
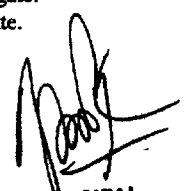
*Explanation of the Grading System:

The University of Delhi Examination system does not have a policy of Grade Point Average (GPA). However, successful candidates are classified on the basis of their performance in Part I, II, and III as per the following:
- First Division: 60% and above in aggregate.
- Second Division: 50% and above, but below 60% in aggregate.
- Third Division: 40% and above, but below 50% in aggregate.

The minimum marks required to pass an examination is 40%

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